EXPLORING THE CALCICOLE – CALCIFUGE GRADIENT IN HEATHLANDS

EXPLORING THE CALCICOLE – CALCIFUGE GRADIENT IN HEATHLANDS

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CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

RISE AND FALL OF DUTCH HEATHLANDS

The history of Dutch heathlands is closely related to the history of human land use. In fact, my family history has been related to heathlands. My grandparents were among the farmers who reclaimed the heathlands in the southern part of the Netherlands during the twenties and thirties of this century. The generation of my parents got used to the idea that the remaining heathlands were worth conserving, and they enjoyed the Sunday morning walks with us in the purple heather reserves. When I grew up, the heathlands were deteriorating more and more, despite their incorporation in nature reserves. It became the subject of my thesis to study the causes of the decline and the restoration of some of the heathland vegetations.

However, the history of the Dutch heathlands comprises a much longer period than the last three generations of my family. About 3000 years B.C. the first Dutch settlers used an agricultural system called 'landnam'. In this system, forests were burnt and the reclaimed land was used for crop production and grazing for several years. When soil fertility was reduced, the fields were abandoned and heathlands developed on the unfertile soils. It is hypothesised by Waterbolk (1954) that the typical dwarf-shrub dominated heathlands were preceded by herb-rich grasslands, which are characteristic of nutrient poor soils. The initial heath vegetation is thought to be a mixture of grass, herb and dwarf-shrub species. Sometimes, the heathlands were used for extensive grazing. However, when inhabitation became scarce and grazing pressure by cattle was reduced, almost all heathlands turned into forest again (De Smidt 1975).

The development of the present heathlands began with the onset of the Middle Ages (450 A.C.). As the population grew and settled, a new agricultural system developed on the sandy soils of the Netherlands. Heathlands made up a crucial part of this system, as they were used for grazing sheep and for the fertilisation of the fields. For the purpose of the latter, sods were cut and put on the floor of the stables where the sheep were kept during the night. When they were drenched with manure, the sods were used to fertilise the arable fields. However, repeated sod-cutting gradually reduced soil fertility in the sod-cut areas, with the result that only plant species adapted to low nutrient availability could survive. Moreover, most of these sandy soils were acidic (6.5<pH<4.5) to acid (pH<4.5), thereby requiring special adaptations for plant growth. These harsh abiotic conditions were suitable for only a number of plant communities, among which the typical heathland vegetation. The heaths were mainly dominated by the dwarf shrubs *Erica tetralix* [1] and *Calluna vulgaris*, although close to the arable fields heathlands rich in grasses and herbs developed (Aerts & Heil 1993). Compared to dwarf-shrub dominated heathlands, these vegetation types are characteristic of soils slightly richer in nutrients.

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[1] Nomenclature of plant species follows Van der Meijden (1996)

It took at least seven to ten years before the heathlands were regenerated to such an extent that sods could be cut again (De Smidt 1975 and references therein), although the frequency of sod-cutting was often reduced to once every 20 to 30 years. This implied that a large area of heathlands was needed to sustain the agricultural system: it is estimated that in 1833, 600,000 ha heathland was found in the Netherlands (De Smidt 1975). This was about 20% of the total surface of the Netherlands. Other authors estimated that about 800,000 ha of heathland existed at the beginning of the nineteenth century (Anonymous 1988). This area has been reduced since 1900, when the use of industrially produced fertilisers became widespread and replaced the use of heath sods. Since then, many of the heathlands have been cultivated, planted with trees for wood production, or have deteriorated as a result of urbanisation, recreation and military use. More recently, atmospheric deposition of N and S and ongoing habitat fragmentation are the main causes for the decline, not only reducing the area of heathland, but also affecting the quality and biodiversity of the remaining heaths. In 1988, only 42,000 ha of heathland were left in the Netherlands, mostly restricted to nature reserves (Anonymous 1988).

HEATHLAND VEGETATION

Typically, heathlands are areas dominated by dwarf shrubs, in which trees and tall shrubs are scarce (Gimingham 1972, De Smidt 1975). However, the exact definition of heathlands has provoked a lot of discussions (De Smidt 1975, Gimingham & De Smidt 1983), concerning either the composition of the vegetation, the origin of the heathlands and the land-use and management.

In this thesis, I return to the practical definition of heaths that was used by the population of the Netherlands for centuries. The Dutch population referred to all extensively grazed common areas as heaths (De Smidt 1975) thus referring to the landscape. Using this definition, heathlands include not only the dwarf shrub dominated areas, but also extensively grazed areas in which grasses and other herbs may be codominant. Despite the floristic and apparent resemblance, bogs are excluded, as they could not be grazed.

At present, the majority of Dutch heathland area is comprised of large areas with rather monotonous dwarf-shrub vegetation (Figure 1). In particular, the dry heathland vegetation type CALLUNO-GENISTION PILOSAE[2], of which *Calluna vulgaris* is the dominant dwarf shrub, may occupy large areas (ca. 23,000 ha; Anonymous 1988). The wet and moist heaths (belonging to the ERICION TETRALICIS alliance), with a dominance of *Erica tetralix,* are far less abundant than the dry heaths, but still occupy rather large areas (about 1,000 ha; Anonymous 1988). The area of other, often species-richer, heath vegetations is even more restricted: these vegetations are either found in gradients between the dwarf-shrub dominated areas and moorland pools, in brook valleys or in dryer habitats where calcareous loam surfaces (Figure 2).

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[2] Nomenclature of plant communities follows Schaminée *et al.* (1995, 1996).

Figure 1 Dwarf-shrub dominated heathland with Juniper (a) and species-rich heath (b) vegetations.

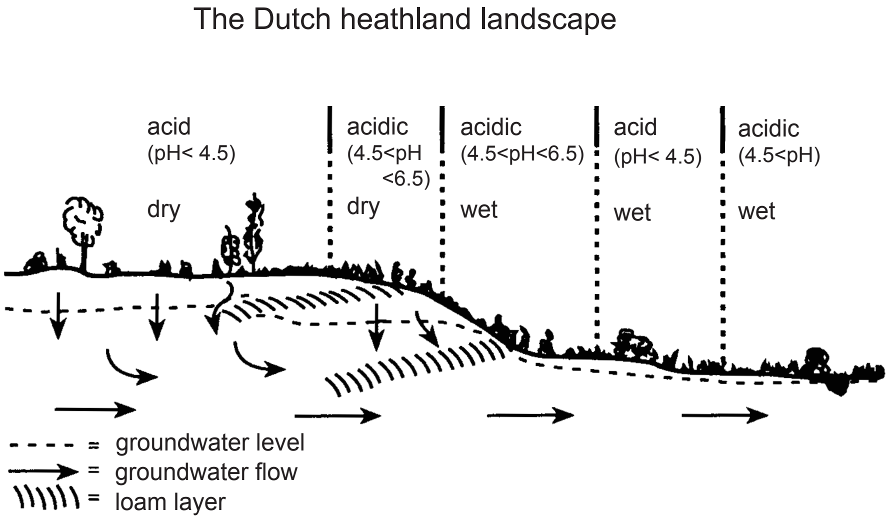

Figure 2 Characteristic habitats of various heath vegetations (After De Graaf *et al.* 1994).

Ecotones from wet heathlands to the species-rich fen meadows in (former) brook valleys (Cirsio dissecti-Molinietum) often contain many species of both communities: e.g. *Succisa pratensis*, *Dactylorhiza maculata*, *Pedicularis sylvatica*, *Cirsium dissectum*, *Platanthera bifolia* and *Carex panicea* are found in combination with characteristic wet heath species such as *Erica tetralix*, *Drosera rotundifolia*, *Gentiana pneumonanthe*, *Narthecium ossifragum*, *Juncus squarrosus* and *Scirpus cespitosus* (Grootjans 1980, Hayati & Proctor 1990, De Graaf *et al.* 1994, Schaminée *et al.* 1995). In habitats where early stages of wet-heath succession are found, such as sod-cut areas, trails and borders of moorland pools, *Erica tetralix* is often observed with *Drosera intermedia*, *Rhynchospora fusca*, *Rhynchospora alba* and *Lycopodium inundatum* (De Graaf *et al.* 1994, Schaminée *et al.* 1995).

On drier soils, species of matgrass swards (Nardo-Galion saxatilis), such as *Arnica montana, Viola canina*, *Nardus stricta*, *Danthonia decumbens*, *Galium saxatile, Polygala serpyllifolia* and *Potentilla erecta* may be present in the heathlands. Often they are accompanied with typical dry heath species such as *Genista anglica* and *Genista pilosa*, and the sedge *Carex pilulifera*. However, under more moist conditions, transitions between the Nardo-Galion saxatilis community, the fen meadow community Cirsio dissecti-Molinietum and wet heath Ericion tetralicis community may also occur (Grootjans 1980; Schaminée *et al.* 1996).

This thesis focuses on species-rich heathlands, thus on the transitions between the dwarf-shrub dominated Calluno-Genistion pilosae and Ericion tetralicis communities and the vegetations of adjacent grasslands, Nardo-Galion saxatilis and Cirsio dissecti-Molinietum communities. I shall refer to these vegetations as **'species-rich heathlands'**. The dry species-rich vegetations can generally be classified as Genisto angli-

CAE-CALLUNETUM subass. DANTHONIETOSUM, GENTIANO PNEUMONANTHES-NARDETUM, or as GALIO HERCYNICI-FESTUCETUM OVINAE communities (Schaminée *et al.* 1996). The latter two associations both belong to the NARDO-GALION SAXATILIS alliance. The wet heath vegetations can be classified as ERICETUM TETRALICIS subass. ORCHIETOSUM, or as LYCOPODIO-RHYNCHOSPORETUM, both belonging to the ERICION TETRALICIS alliance (Schaminée *et al.* 1995).

ECOLOGY OF HEATHLANDS

Environmental and vegetational processes determine the actual vegetation. Climate, hydrology, geology, land use and succession are generally recognised as important factors in the establishment of vegetations. The effect of these factors on the heathland vegetation is generally as follows:

- *Climate.* A mild Atlantic climate with a relatively high air humidity and mild winters is a prerequisite for atlantic heathlands (Gimingham 1972). Mean daily temperatures rarely exceed 20 °C. Such conditions are found in all parts of the Netherlands.
- *Hydrology.* Wet and dry heath vegetations have very distinct characteristics. Dry, *Calluna* dominated heathlands develop on freely drained soils, where groundwater tables are usually far below the rooting zone (Gimingham 1972). The influence of seepage or lateral moving groundwater is absent. In wet heathlands groundwatertables may vary considerably among the various vegetation types (Jansen 2000). In general, the soil moisture content of the rooting zone is high during autumn, winter and early spring, due to groundwater levels reaching to or above soil surface. This may either originate from seepage or from lateral groundwater flow into the rooting zone, or it may arise from the stagnation of rain water above an impermeable soil layer. In early summer the groundwater levels quickly fall; groundwater levels of 130 cm below the soil surface have been recorded (Jansen *et al.* 2000), but mostly groundwater levels remain closer to the surface (within c. 1 m; Niemann 1973, Jansen *et al.* 1996, Jansen 2000). As the water quality of the three water types influencing the rhizosphere may vary greatly, the effects of hydrology extend further than the soil moisture content. Soil chemical and biological processes are affected by both the quality of the water and the soil moisture content (aerobic vs anaerobic conditions), and hence hydrology plays an important role in nutrient availability and soil acidity in wet heathlands (Jansen 2000).
- *Geology.* In general, heaths are found on sandy or loamy soils, which mostly have developed a podsolic profile (Gimingham 1972). Due to the fact that precipitation exceeds evapotranspiration, most of these soils are acid to slightly acidic. pH values in the top soil layers generally vary from 3.4 to 6.5 (Gimingham 1972, Hayati & Proctor 1990, Houdijk *et al.* 1993, De Graaf *et al.* 1994). The acidity of the soil is a key factor in the availability of nutrients to plants. Furthermore, soil pH, or rather the buffering processes affect the concentrations

of potential phytotoxic metals as aluminium, manganese and iron. The acid soils, with pH < 4.5, are mainly buffered by the dissolution of aluminium (hydro)oxides (Scheffer & Schachtschabel 1979), resulting in high Al^{3+}-concentrations (Kinraide 1997). Cation exchange processes at the negatively charged soil adsorption complex (clay particles or organic matter) buffer the slightly acidic (pH 4.5-6) soils against acidification. Due to the exchange of protons by calcium, magnesium, potassium and sodium, the availability of these cations to plants is usually high, in contrast to their availability in the more acid, aluminium-buffered soils. In soils where cation exchange processes are the main buffering process, Al^{3+}-concentrations remain low. A third buffering mechanism, weathering of silicates, occurs at soil pH 5-6.5. However, as silicate weathering is very slow, it is of less importance to ecological processes (Scheffer & Schachtschabel 1979). Thus the dissolution of aluminium-(hydro)oxides and cation exchange processes are the main buffering processes in heathland soils. In addition to the acidity of the soil, the general soil characteristics also determine the waterholding capacity of the soil, thereby affecting soil moisture content, nutrient availability and redox potential of the soil.

- *Succession and land use.* In the Netherlands, inland heathlands are not a climax vegetation. Sooner or later, trees and shrubs will invade the heathlands, unless they are managed by man (Gimingham 1979, Gimingham & De Smidt 1983, Aerts & Heil 1993). In fact, due to the management of the Dutch heathlands, natural succession is greatly inhibited. Widespread management methods as mowing, grazing, burning and sod-cutting (the removal of vegetation and litter layers) all have an impact on both soil processes (especially on nutrient availability) and on biological processes (Gimingham & De Smidt 1983). Regarding the latter, gap formation, which enables germination of plants, competition and succession, is of special interest.

Despite the fact that hydrological and geological processes, together with land use, eventually determine the vegetation characteristics, individual plant establishment and survival is determined by processes in the rhizosphere, the availability of light and water, and the temperature. As briefly mentioned before, especially the nutrient availability, the acidity of the soil and the soil moisture content play a major role in the establishment, development and survival of the heathland species.

NUTRIENT AVAILABILITY

Nutrient availability is low in all heathland communities. Nearly all species have symbiotic relationships with mycorrhizal fungi, which facilitate the uptake of the (scarce) nutrients. The dwarf-shrubs are infected with specific ericoid mycorrhizas, whereas vesicular-arbuscular mycorrhiza (VAM) infections are common in other species (Heijne 1995, Smith & Read 1997). It is generally assumed that nutrient uptake, especially of phosphorus, is increased by mycorrhizal infections, although the nature of this relationship is not yet fully understood. Furthermore, mycorrhizal infections increase plant resistance to plagues, drought and toxic substances (e.g. Smith & Read 1997).

In the past, the dwarf-shrub dominated communities were probably mostly limited by nitrogen (Gimingham 1972, Bobbink *et al.* 1992, Aerts & Heil 1993), though limitation of phosphorus has also been suggested (Gimingham 1972). However, with the present increased atmospheric nitrogen deposition, up to 40 kg N ha^{-1} yr^{-1} in the Netherlands (Bobbink & Heil 1993), it is thought that some heathlands are no longer limited by N (Aerts & Heil 1993, Bobbink *et al.* 1998, Lee & Caporn 1998). Some studies suggest that the original N-limitation may have changed into P-limitation (Aerts & Berendse 1988, Prins *et al.* 1991, Carroll *et al.* 1999).

Little is known about nutrient limitation in species-rich heathlands, but from the few studies on this subject, a complicated picture can be drawn. Experiments by Hayati & Proctor (1991) showed that in wet species-rich heathlands, nutrient limitation may vary between species and sites. Moreover, limitation by P and N may vary in time in the closely related fen meadows (Grootjans *et al.* 1986). Limitation by K has also been observed in some fen meadows (Pegtel 1983).

SOIL ACIDITY: THE CALCICOLE - CALCIFUGE GRADIENT

The importance of the top soil acidity in the distribution of heathland species has been emphasised in many studies (Balme 1956, Hayati & Proctor 1990, Houdijk *et al.* 1993, De Graaf *et al.* 1994, Roelofs *et al.* 1996). It has been observed that the species-poor, dwarf-shrub dominated heaths are found on acid soils, with pH values generally below 4.5 (Matzner 1980, Hayati & Proctor 1990, Houdijk *et al.* 1993, De Graaf *et al.* 1994). Characteristic species of the species-rich heath vegetations are restricted to soils with higher pH values, mainly between 4.5 and 6 (Figure 3; Hayati & Proctor 1990, Houdijk *et al.* 1993, Roelofs *et al.* 1996). The difference in species distribution along a soil pH gradient is often referred to as the calcifuge-calcicole gradient (e.g. Balme 1956, Hayati & Proctor 1990). In such a gradient, the calcifuge species are found on acid soils, whereas the calcicole species are restricted to moderately acidic soils or soils with an even higher pH.

It is, however, questionable to what extent this distinction in distribution is simply a matter of pH (Rorison 1986, Runge & Rode 1990, this thesis). In water culture experiments, it has been shown that many species, including those characteristic of species-rich heaths, resisted pH values of 4.0-4.5 (Van Dobben 1991). This strongly suggests that the pH itself is not the main determining factor in the distribution of the heathland species, as the species of the less acid habitats could also tolerate low pH values.

The marked difference in species distribution around pH 4.5 does, however, coincide with the distinction in the two major buffering processes in the soil, the alu-

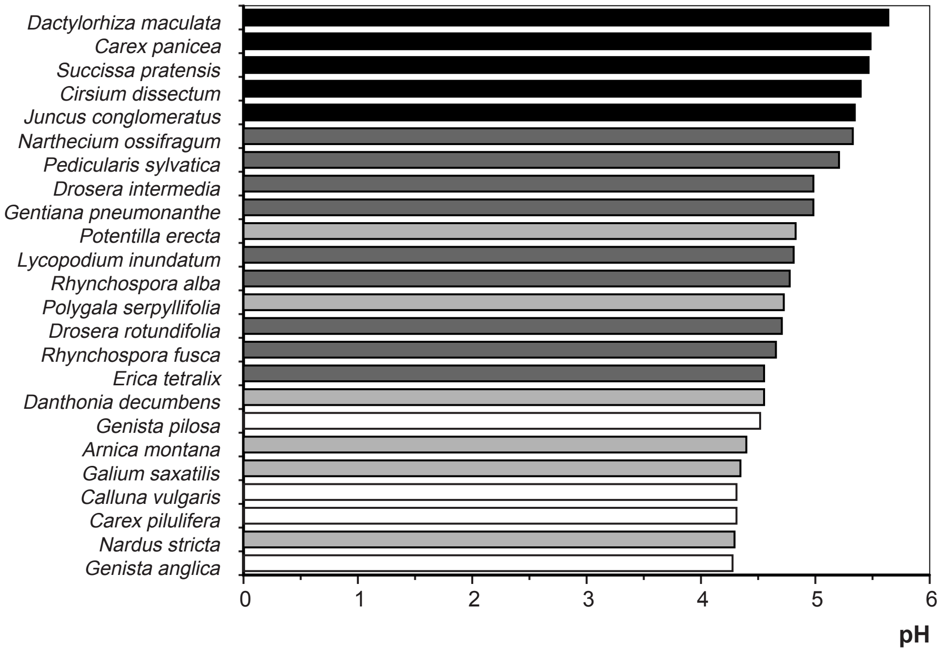

Figure 3 pH_{H2O} (median values) of characteristic species in Dutch heathland vegetations. Black: *Cirsio dissecti-Molinietum*, dark grey: *Ericetum tetralicis*, grey: *Nardo-Galion saxatilis*, white: *Callunetum-Genistion pilosae.* N>10, except for *Polygala serpyllifolia* (n=5) and *Genista pilosa* (n=7).

minium buffering (pH<4.5) and cation exchange buffering (pH 4.5-6; Scheffer & Schachtschabel 1979). At pH values below 4.5, the availability of macronutrients such as calcium, magnesium and potassium strongly decreases, whereas the concentrations of aluminium, and micronutrients such as manganese and iron increases.

Moreover, soil pH is known to affect soil microbiological processes such as decomposition, mineralisation and nitrification (Swift *et al.* 1979), which may change the nitrogen economy considerably. Both the quantity of available mineral nitrogen and its form, e.g. ammonium or nitrate, are (partly) dependent on soil pH. Low decomposition rates in general and low N mineralisation rates specifically, limit the availability of mineral nitrogen to plants in acid soils. Due to the inhibition of autotrophic nitrification at low pH values, ammonium is the dominant mineral nitrogen form in acid heathland soils (Roelofs *et al.* 1985, Troelstra *et al.* 1990). In contrast, in slightly acidic and circumneutral soils, nitrate is the dominant mineral nitrogen form, as was shown for Swedish forest soils (Falkengren-Grerup 1995). As many plant species prefer one mineral nitrogen source over the other, or a mixture of nitrate and ammonium (Gigon & Rorison 1972, Falkengren-Grerup & Lakkenborg-Kristensen 1994), such differences in dominant mineral N form are likely to have a great impact on the distribution of plant species.

The characteristic species of species-poor heaths are adapted to acid soil conditions. They tolerate high aluminium concentrations (Hackett 1965, Rorison 1985) and generally prefer ammonium over nitrate as nitrogen source (Hackett 1965, Rorison 1985, Troelstra *et al.* 1995). Such information on the characteristic species of species-rich heathlands is scarce. Fennema (1992) found that *Arnica montana* was only found on soils with low aluminium concentrations. Sites where the species had recently become extinct had significantly higher aluminium concentrations, thus suggesting that the species is sensitive to high aluminium concentrations. Moreover, Heijne (1995) showed a negative correlation between the performance of *Arnica* and the aluminium concentration in the soil. His experiments showed that not only plant growth was reduced under high aluminium concentrations, but also that mycorrhizal infection rates became decreased. However, in water culture experiments, *Arnica montana* proved to be rather tolerant to high aluminium concentrations (Pegtel 1987, Kroese *et al.* 1989). These experiments were however carried out with high nutrient solutions. As aluminium toxicity initially affects the root system and concomittantly nutrient uptake will be hampered, high nutrient solutions may reveal the toxic effects of aluminium on plant growth.

With regard to the nitrogen source, Pegtel (1994) showed that ammonium nutrition in *Arnica montana* resulted in a decreased potassium uptake, suggesting that the species is not adapted to ammonium nutrition. This is in agreement with the results of 2 year fertilisation experiments by Heijne *et al.* (1992), who found that both plant growth and mycorrhizal infection were reduced after application of 90 kg ha^{-1} $(NH_4)_2SO_4$. Yet, in short-term pot experiments, no effects of ammonium on *Arnica montana* were observed (Heijne *et al.*1994).

THE RECENT DECLINE OF THE HEATHLAND SPECIES AND VEGETATIONS

Unfortunately, the decline of the heathlands did not stop with their incorporation in nature reserves. Atmospheric pollution, changes in local and regional hydrology, habitat fragmentation and the lack of adequate management all contributed to the further deterioration of the heathlands (Anonymous 1988). Atmospheric deposition of N and S has had a large impact on the nutrient-poor, poorly buffered soils, due to its acidifying and eutrophicating effects. Deposition of nitrogen may drastically improve nitrogen availability, either directly or indirectly through ecosystem changes (e.g. Roelofs 1986, Berendse 1990, Van Vuuren 1992, Aerts & Heil 1993, Bobbink *et al.* 1998, Lee & Caporn 1998).

In the species-poor heaths, this has led to a change in vegetation from dwarf-shrub dominated vegetations into monotonous grasslands, which are either dominated by *Molinia caerulea* (on former wet and dry heaths) or *Deschampsia flexuosa* (on dry heaths). From the comprehensive work on these vegetational transformations, it has become clear that it is not only a matter of changed competition between the dwarf shrubs and grasses. In fact, the ecosystem is affected in many aspects, including plant growth and reproductive capacities, sensitivity to plagues of heather beetles, frost and drought, mycorrhizal relations, decomposition, N-mineralisation and eventually competition (see Aerts & Heil 1993, Uren *et al.* 1997, Power *et al.* 1998, Carroll *et al.* 1999).

It is not certain to what extent a similar process has contributed to the decline of the species-rich heathlands and matgrass swards. Dueck and Elderson (1992) performed competition experiments with *Arnica montana*, *Viola canina* and the grass *Agrostis canina*, under different N and S deposition treatments. They concluded that acidification was more likely to cause primarily the decline of the herbs, although changed competition in favour of *Agrostis* might add to the decline.

To plant species that are not adapted to acid conditions (pH<4.5), as are the characteristic species of the species-rich heaths, soil acidification is likely to be harmful. In fact, Van Dam *et al.* (1986) found a close correlation between the decline of many plant species of cation-buffered soils, among which some species of species-rich heathlands, and acidifying S deposition. The importance of high aluminium concentrations, probably resulting from recent soil acidification, in the decline of *Arnica montana* has been shown by Fennema (1992). Another indication of the important role of acidification in the decline of species-rich heathlands comes from the work of Roelofs *et al.* (1996; Figure 4). In their field survey on *Arnica montana*, it was shown that *Arnica* appeared on soils with much lower Al/Ca ratios than the species-poor heaths. Furthermore, ammonium concentrations in poorly developed *Arnica* populations were almost three times as high as those in well developed populations; in poorly developed populations ammonium concentrations equalled those in the *Calluna* dominated species-poor heath vegetation. It is known that (high) ammonium concentrations may be toxic to many plant species (Mehrer & Mohr 1989). Furthermore, the uptake of ammonium instead of nitrate, or an unbalanced mixture of both, may cause nutritional imbalances in the plant. It is thought that these processes play an important role in the decline of West European forests (Nihlgård 1985, Roelofs *et al.* 1986, Van Dijk 1993).

Thus, the results of field surveys strongly suggest that soil acidification causes the decline of species-rich heathlands. However, physiological knowledge on the aluminium tolerance, as well as the preference for mineral nitrogen sources of plant species from these species-rich heathlands, is scarce, whereas knowledge on the interactions between aluminium and nitrogen source is almost lacking. Such knowledge is however necessary if one is to gain a better understanding of the causes for the decline of these rare plant communities.

AIM OF THE THESIS

This thesis focuses on the interaction between soil factors and the vegetation of heathlands, in order to gain a better understanding of the distribution of different heath vegetations and species along the calcicole - calcifuge gradient. Special attention will be paid to potential phytotoxics such as aluminium and ammonium, as

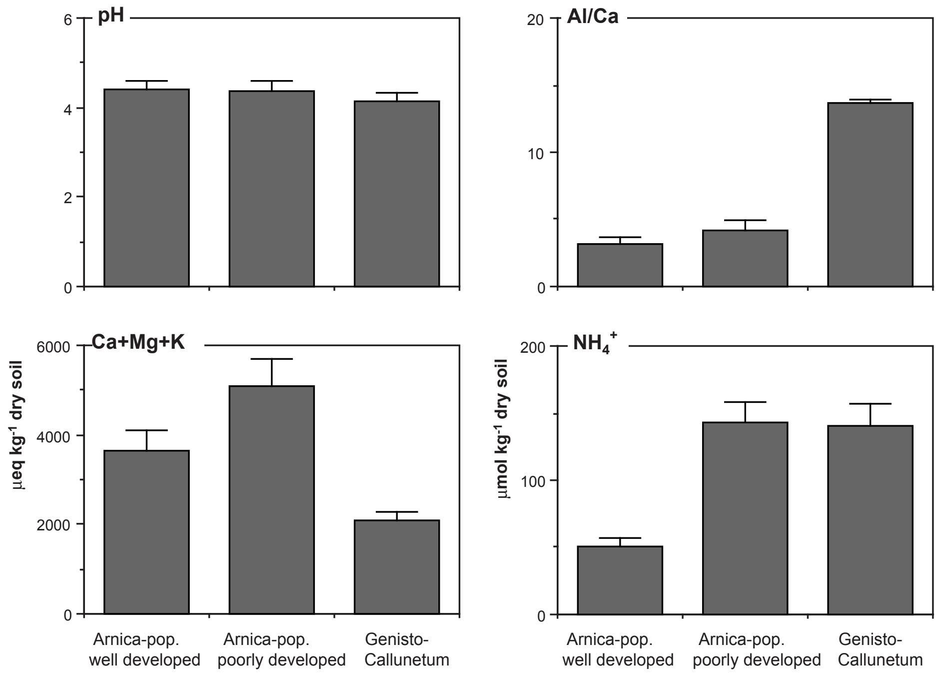

Figure 4 Soil pH, Al:Ca ratios (in mol mol^{-1}), NH_4^+ concentrations (µmol kg^{-1} dry soil) and base cations concentrations (µeq kg $^{-1}$ dry soil)in well developed *Arnica montana* populations (n=8), poorly developed *Arnica montana* populations (n=13) and dry, *Calluna vulgaris* dominated heaths (n=48). Samples were taken in Dutch heathlands in 1993 and 1994. pH, Al:Ca ratio and NH_4^+ have been measured from water extracts, whereas the base cation content is the sum of exchangeable 2*Ca^{2+}, 2*Mg^{2+} and K^+ concentrations (after Roelofs *et al.* 1996)

well as to nitrogen nutrition, which may be an important factor in both the distribution of heathland species along the calcifuge-calcicole gradient and the deterioration of the species-rich heaths. Furthermore, I shall explore the possibilities of restoring deteriorated dry heath ecosystems.

OUTLINE OF THE THESIS

In this introduction, I have briefly discussed the heathland landscape, its different vegetation types and their relations with hydrology, soil chemistry and management. However, a consistent part of this knowledge comes from studies which have been performed on different sites and/or in different countries, hence under different climatic, geological and hydrological conditions. Moreover, the experiments have been carried out in order to answer different research questions, using different methods. So, the information on ecosystem functioning of heathlands, in particular of species-rich heathland communities, is scattered and fragmented, and shows some serious gaps. Therefore, two wet heathland vegetations have been closely examined throughout the year (Chapter 2). The two types, one species-poor, *Erica tetralix* dominated heath and one species-rich heath vegetation were only a few metres apart, thus minimizing other than stand related variance. In both vegetation types top-soil chemistry, nitrogen mineralisation and hydrology were studied in order to find the key factors in the functioning of the two ecosystems. The effects of management on the vegetation have also been discussed.

The chapters 3, 4 and 5 have a different approach: in these chapters, ecophysiological studies are presented on the effects of the nitrogen source (chapter 3) and aluminium concentrations (chapter 4) on three heathland species. The effects of high ammonium concentrations are also studied in chapter 3. Such high ammonium concentrations may arise from the present atmospheric ammonium deposition rates in the Netherlands. Chapter 5 considers the possible interactions between the nitrogen source and aluminium concentration. The ecophysiological studies are all carried out with *Arnica montana* and *Cirsium dissectum*, which are characteristic of dry and wet species-rich heaths respectively. Their responses are compared with those of the acid-tolerant dwarf-shrub *Calluna vulgaris* in the chapters 3 and 4. Although it is difficult to link these studies directly to field conditions, they do provide a better insight in the physiological mechanisms by which the characteristic species of both heathland vegetation types function under the differing abiotic conditions at the acid extreme of the calcifuge-calcicole gradient.

The knowledge gained from the previous chapters is used to develop adequate restoration measures for different deteriorated heathland communities (chapter 6). As it is shown that soil acidification is likely to cause the decline of the species-rich heathlands, liming has been used to counteract this process. However, as the possibility of nitrogen eutrophication cannot be ruled out, a factorial experiment of liming and sod cutting was set up. Apart from the removal of excess nitrogen, sod cutting also has the advantage of creating a bare substrate, which facilitates the germination and establishment of species. The population biology of *Arnica montana* in response to the various restoration measures has been studied in detail.

In the final chapter (7), the responses of the heathland species to aluminium and nitrogen nutrition in relation to the distribution along the calcifuge-calcicole gradient in heathlands are considered. Furthermore, the importance of soil acidification, due to either atmospheric deposition of N and S, or to changes in the hydrology, in the decline of species-rich heathlands will be discussed. The thesis is concluded with a discussion on the perspectives for restoration of the characteristic heathland vegetation.

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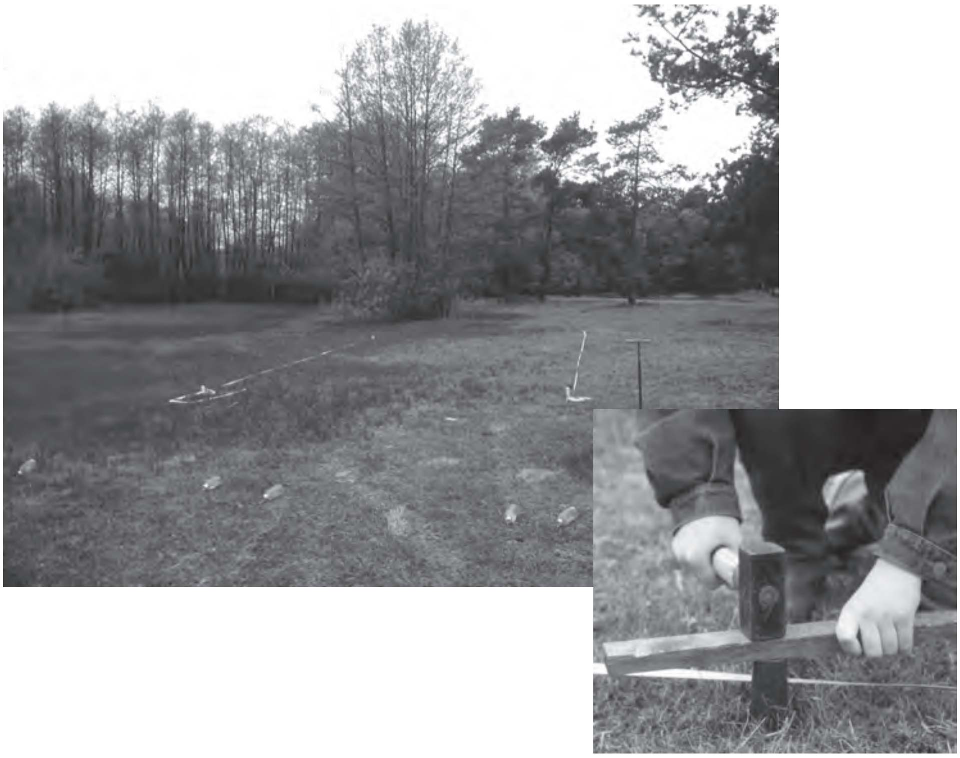
CHAPTER 2

ECOSYSTEM FUNCTIONING OF TWO WET HEATH VEGETATIONS ALONG A CALCICOLE – CALCIFUGE GRADIENT



WITH ROLAND BOBBINK & JAN G.M. ROELOFS

ECOSYSTEM FUNCTIONING OF TWO WET HEATH VEGETATIONS ALONG A CALCICOLE – CALCIFUGE GRADIENT

ABSTRACT

Vegetation patterns in wet heathlands related to differences in soil pH can be described as calcicole – calcifuge gradients. In mire ecosystems, differences in hydrology often underly the calcicole – calcifuge gradient. For wet heath vegetations, such a relation is less established. Therefore, hydrology and top-soil chemistry in a well developed wet heath calcicole – calcifuge gradient have been monitored closely throughout a year. It has been shown that the species-poor dwarf-shrub dominated heath established itself on more acid soils (pH < 4.5) than the species-rich heath (pH ± 5.5). The latter vegetation received calcareous groundwater into the rooting zone for a significantly longer period each year than the species-poor wet heath. Presumably, the calcareous groundwater recharges the soil adsorption complex in the species-rich heath with enough base cations to withstand further acidification, whereas the supply of base cations to the species-poor heath is not sufficient to buffer the top soil above pH 4.5.

As vegetation patterns in heathlands are known to show differences in nutrient availability, especially of nitrogen, the availability of ammonium and nitrate in the vegetation gradient received special attention. Furthermore, N-mineralisation and nitrification rates have been determined in the different heath vegetations. The observed mineralisation and nitrification rates were rather low for mature Dutch heath vegetations. It is suggested that the low mineralisation rates are caused by the intensive management of the studied site. This prevents organic matter from accumulating, thereby reducing the available substrate for mineralisation.

The results are discussed in relation to plant distribution, as well as in relation to perspectives for conservation of these endangered ecosystems.

INTRODUCTION

Wet heathlands occur in regions with an atlantic climate, and nutrient-poor, acidic soils, with high water tables at least a part of the year. The landscape consists of various vegetation types, with the dwarf-shrub *Erica tetralix* usually being the dominant plant species (Gimingham & De Smidt 1983). The dwarf-shrub dominated areas are alternated with sites in which the grasses *Molinia caerulea* or *Nardus stricta* may be highly abundant or even dominant. Extensive research on the dominance of the grass *Molinia caerulea* in heathlands has revealed that the grass heaths have a higher availability of nitrogen than the dwarf-shrub dominated heaths (Aerts & Heil 1993 and references therein).

In transitions from wet heaths to valley bogs or fen meadows, patches are found with a relatively high abundance of herbaceous species as *Narthecium ossifragum*, *Cirsium dissectum*, *Gentiana pneumonanthe*, *Succisa pratensis* and *Dactylorhiza maculata* (Loach 1966, Grootjans 1980, Gimingham & De Smidt 1983, Hayati & Proctor 1990, Boeye & Verheyen 1994, Roelofs *et al.* 1996, Jansen *et al.* 1996). The latter gradient has been referred to as a calcicole - calcifuge gradient (Balme 1956, Hayati & Proctor 1990), as the distribution of the species coincided with distinct differences in top-soil chemistry related to the soil pH. The calcifuge, dwarf-shrub dominated heaths are restricted to the acid soils (pH usually below 4.5), where calcium and magnesium concentrations are low. In contrast, the calcicole wet heath vegetations, are found on less acid soils (pH 4.5-6.0), with higher base cation concentrations. A survey on the distribution of individual species along this gradient in the Netherlands has shown that, although soil aluminium concentrations do not always differ between the calcifuge and calcicole species, the Al/Ca ratio is significantly higher in the rhizosphere of calcifuge species (Houdijk *et al.* 1993b).

All studies on the calcicole - calcifuge wet heath gradient have demonstrated variation in the concentrations of the major nutrients N and P (Balme 1956, Hayati & Proctor 1990, Houdijk *et al.* 1993b), but a principal component analysis by Hayati and Proctor (1990) showed that they were less important in explaining the species distribution than pH and base cation concentrations. In general, both mineral P and N concentrations are low in these vegetations. More specific, the P contents of the soil are lower in the calcicole heaths than in the calcifuge heath. Measurements on the N concentrations were less consistent, as ammonium concentrations (exchangeable fraction) were highest in the calcifuge heath in the study by Hayati & Proctor (1990), but showed an opposite relation with the species distribution in the survey by Houdijk *et al.* (1993b). Nitrate concentrations (only determined by Houdijk *et al.* 1993b) were always low.

These differences in the concentrations of nutrients and other minerals are the result of hydrological, biogeochemical and biological processes that occur in the upper soil layers. Plant uptake and immobilisation by microorganisms are important output factors for nutrients. The output is balanced by input by groundwater and atmospheric deposition and by mineralisation of organic matter.

The importance of hydrological processes is evident in calcicole - calcifuge gradients in heathlands (Jansen *et al.* 2000) and in mire ecosystems, which bear a close resemblance to the gradients in heathlands (Wassen *et al.* 1989, Boeye & Verheyen 1992, Jansen & Roelofs 1996). It has been shown that differentiation between fens and bogs, is mainly due to differences in hydrology and hydrochemistry, with the upper peat layers of bogs depending strongly on rain water, whereas fens are influenced by rain water and calcareous ground- or surface water (Wassen *et al.* 1989, Lamers *et al.* 1999). This causes great differences in the top-soil chemistry, with acid conditions in the bogs whereas the fen soils have higher pH values and concentrations of base cations (Boeye *et al.* 1994, Beltman *et al.* 1996). We hypothesise that in wet heathlands, hydrological processes may play a similar role in the distribution of species along the calcicole - calcifuge gradient, as calcareous groundwater reaches into the rooting zone in these ecosystems for a considerable period each year.

Though nutrient availability is less important in the calcicole - calcifuge heathland gradient than pH-related factors (Hayati & Proctor 1990), it does explain some of the distribution of the species. In general, nutrient availability in ecosystems is, apart from natural in- and output of nutrients, mainly dependent on decomposition and mineralisation processes. However, these processes are strongly affected by environmental conditions such as pH, soil moisture content and aeration (Swift *et al.* 1979), and by the quality of the litter (Swift *et al.* 1979, Heal *et al.* 1997).

With respect to nitrogen, the last phases of the decomposition process, e.g. mineralisation and nitrification, have been studied extensively in acidic heathlands (Roelofs *et al.* 1985, Berendse *et al.* 1987, Berendse *et al.* 1989, Berendse 1990, De Boer 1989, Van Vuuren *et al.* 1992, Morecroft *et al.* 1994). Many of these studies focussed on differences in dwarf-shrub and grass dominated heathlands (Berendse *et al.* 1987, Berendse *et al.* 1989, Berendse 1990, De Boer 1989, Van Vuuren *et al.* 1992). They have shown that the quality of the litter was greatly affected by the species composition of the heath, whereas the litter quality itself did affect N mineralisation rates (Van Vuuren *et al.* 1992), resulting in higher mineralisation of nitrogen in the grass heaths than in the dwarf-shrub dominated heathlands. In other words, species composition affects N mineralisation rates in heathlands.

Moreover, it has been shown in heathland soils (Roelofs *et al.* 1985) that nitrification rates decrease with decreasing pH and that, as a result, ammonium accumulates, whereas nitrate concentrations become increasingly low. Hence, not only the amount of mineral nitrogen available to plants is affected by the heath ecosystem, also the dominant mineral N form will be affected along the calcicole - calcifuge gradient. This will in turn affect the distribution of species, as it has been shown that many heathland species favour either ammonium or nitrate as their nitrogen source (Gigon & Rorison 1972, Falkengren-Grerup & Lakkenborg Kristensen 1994, Troelstra *et al.* 1995, De Graaf *et al.* 1998).

At present, the wet heathland ecosystem in Western Europe is seriously threatened. Both the characteristic vegetation types as well as the species have been declining during the last decades (De Smidt 1975, Anonymous 1988, Tickle *et al.* 1995). The characteristic species and plant communities of the calcicole wet heaths are particularly becoming increasingly rare as a result of soil acidification and hydrological changes. Knowledge of the functioning of the wet heathland ecosystem as a whole, as well as of the important processes and mechanisms, may help to conserve this ecosystem. However, such knowledge of calcicole wet heaths is scarce. Moreover, studies which relate the species distribution to top-soil chemistry, as well as to hydrology and N mineralisation, do not exist. Therefore, we performed an ecosystem study in the

Breklenkampse Veld, a Dutch wet heathland, in order to elucidate the determining factors in the distribution of the calcifuge-calcicole wet heath vegetation.

The study is based on the assumption that differences in concentrations of minerals and nutrients in the upper ten centimeters of the soil, to which the major part of the plants roots are confined, cause the distinct borders between the various plant communities. As the calcicole - calcifuge gradient extends only over twenty meters, differences in nutrient concentrations due to atmospheric deposition are highly unlikely. We closely monitored top-soil chemistry, groundwater fluctuations and groundwater quality. In addition, we related these factors to each other in order to gain more insight in the relation between hydrology and biogeochemical processes in the rooting zone. We hypothesise that the calcicole - calcifuge gradient in wet heathlands is based on differences in hydrology, particularly in the influence of calcareous groundwater in the rhizosphere.

Furthermore, we studied N mineralisation and nitrification, as we hypothesise that, as soil pH has a major impact on species composition of heathlands, it might also influence net N mineralisation and nitrification rates in heathland vegetations other than the species-poor types found on acid soils. Soil pH might either directly affect the mineralisation processes, or it may have an indirect effect via different qualities of litter due to the different species composition of the two zones.

The results of the study will be discussed in relation to the deterioration of wet heathlands and the possibilities for conservation.

MATERIAL AND METHODS

Site description

The experiment has been carried out in the Dutch nature reserve 'Breklenkampse Veld' (52°26'N, 7°01'E), owned and managed by the 'Overijssels Landschap'. The Breklenkampse Veld is an undulating area, which developed during the Pleistocene, when the sandy top soils were deposited after the last ice age. During the warmer and wetter Holocene, thin layers of loam were deposited in the lower parts of the reserve by the precipitation of loam particles during periods of inundation. Later, peat developed in these lower parts. The thickness of the peat layer varies from ten to over fifty cm; the layer is thinner (5-8 cm) in the experimental plots.

The Breklenkampse Veld consists of a mosaic of forests, wet and dry heaths and nutrient-poor grasslands. A zonation in different grass and heath vegetations is found, with the grass vegetations in the lower parts and the dwarf-shrub dominated vegetations in the higher and drier areas. The boundaries between the different vegetation types are sharp, probably as a result of differences in abiotic conditions. In the reserve, heath vegetations have developed on podzolised soils, whereas the wet grasslands are mainly found on the peaty soils.

The Breklenkampse Veld is a rather intensively managed nature reserve: the grasslands are mown annually, whereas nowadays, the heaths are mown every three years. The hay is always removed.

Experimental design

From 28-4-1995 until 2-4-1996, an experiment was carried out in order to determine the main processes that affect the functioning of two wet heath vegetations. Two transects of 12 m were laid out along the height gradient. The upper transect (referred to as 'species-poor zone', width 5 m) was laid out in a dwarf-shrub dominated heath, in which *Calluna vulgaris* and *Erica tetralix* were the dominant species. In the lower transect ('species-rich zone', width 1-2 m), *Potentilla erecta*, *Pedicularis sylvatica* and *Succisa pratensis* were the most abundant species (Table 1). This zone can be characterised as a transitional stage between the heath and grassland vegetation. The 'heart to heart' distance between the two zones was 8 m; the soil surface of the species-rich zone was situated approximately 11 cm below the surface of the species-poor zone.

Both transects were last mown in August 1994. During the experiment (1995-1996), management was carried out as usual, meaning that in August 1995 the lower zone was mown, whereas the species-poor zone was not mown that year. Mowing was performed by hand in the experimental plot, without disturbing the experimental equipment for the determination of mineralisation and nitrification.

VEGETATION

Early July 1995, the vegetation was recorded in three 1 m^2 plots per zone, using the Braun-Blanquet scale. As there was hardly any difference in species composition, nor in species abundance between the plots per zone, the species composition is given per zone.

HYDROLOGY

Fluctuations in groundwater level were recorded every other week in the piezometer (installed in 1990; filter depth: 2 m below soil surface). One piezometer was used for both zones. The piezometer was located approximately 12 m outside the experimental plot, at a level similar to that of the species-rich zone. Soil surface heights of the vegetation zones and the piezometer were carefully measured in August 1995. This data and the data on groundwater levels were used to calculate the number of days the groundwater reached the rooting zone of both vegetation zones. The rooting zone is defined as the upper 10 cm of the soil, as the major part of the roots is confined to this zone.

Table 1 Vegetation of the two experimental heathland zones in Breklenkamp. Abundance of species is recorded after Braun-Blanquet. Seedlings of trees are omitted.

	Species-poor zone	**Species-rich zone**
Total vegetation cover	27%	70%
Moss cover		40%
Erica tetralix	3-5%	
Calluna vulgaris	3-5%	
Molinea caerulea	8%	+
Carex panicea	1	1
Juncus acutiflorus	r	r
Potentilla erecta	+	25%
Juncus squarrosus	r	
Salix repens	+	r
Scirpus cespitosus	r	
Festuca ovina		5%
Pedicularis sylvatica		8%
Succisa pratensis		8%
Anthoxanthum odoratum		1
Holcus lanatus		1
Lotus pedunculatus		+
Lythrum salicaria		+
Hydrocotyle vulgaris		1
Carex nigra		+
Agrostis capillaris		+
Potentilla palustris		+
Equisetum palustre		+
Ranunculus flammula		r
Viola palustris		r
Luzula campestris		r
Lysimachia vulgaris		r
Cirsium palustre		r

The quality of the shallow groundwater was determined from samples taken from the piezometers in November 1995, January 1996 and April 1996. Prior to sampling, the tube was emptied and allowed to refill with groundwater again. In the laboratory, subsamples of the groundwater were immediately analysed for pH and HCO_3^- (by titration with HCl to 4.2). The remains of the samples were frozen (-18 °C) in polyethyleen pots until further analyses for NH_4^+, NO_3^-, Ca, Mg, K, Na, Al, Fe, Zn, Mn, Cl, S and P. For analysing methods, see the section ‘extraction methods and chemical analyses’.

Top-soil chemistry

During the experimental period, samples of the top soil (top 10 cm, ∅ 2.5 cm, after removal of vegetation and litter) were taken every 4 weeks, from April to November 1995. In winter and early spring, soil samples were taken at larger intervals (January and April). We took 10 replicates per vegetation zone per sampling time. Samples were stored individually in polyethylene bags at 4 °C until further analysis.

Both water-extractable and exchangeable nutrient concentrations were determined (exchangeable fractions are indicated by the suffix ‘$_{ex}$’, waterextractable fractions by the suffix ‘$_{aq}$’). pH $_{aq}$, $NO_{3\ aq}^{-}$, $NH_{4\ aq}^{+}$, $NH_{4\ ex}^{+}$ and soil moisture content were measured in all samples. Ca_{aq}, Ca_{ex}, Mg_{ex}, K_{ex}, Al_{aq}, Fe_{aq} and P_{aq} were determined less frequently, as we expected them to be less affected by seasonal processes. Bulk density was estimated in the April ’95-samples. Organic matter content (measured by loss of ignition (4 hours at 550 °C)), total C and total N were all measured in the samples taken in November 1995.

Net N mineralisation and nitrification

Net N mineralisation and net nitrification were determined by an *in situ* method. On all sampling dates, except for April 1996, additional soil cores (N=10) were taken for the determination of N mineralisation and nitrification. These soil cores were taken pair-wise with the soil samples taken with the auger for determination of top-soil chemistry.

Soil cores for the determination of mineralisation were taken using pvc tubes (length 15 cm, internal diameter 3.4 cm, wall thickness 0.3 cm). They were removed from the tubes, after which vegetation and litter were removed, and the soil cores were cut off at 10 cm. Next, the soil cores were carefully inserted into the tubes again and capped at the bottom to prevent leaching of minerals. Tubes were also capped at the top: five holes (∅ 1 mm) were made in the top cap in order to allow gas exchange. The capped soil cores were re-inserted into the soil and left there until the next sampling date. Then the cores were collected and transported to the laboratory, where $NH_{4\ ex}^{+}$ and $NO_{3\ aq}^{-}$-concentrations were determined.

Net N mineralisation is defined as the increase in mineral N concentrations ($NH_{4\ ex}^{+-}$-N + $NO_{3\ aq}^{-}$-N) between the paired inital and the incubated samples; likewise, net nitrification is the increase in $NO_{3\ aq}^{-}$-N-concentrations. Both net N mineralisation and net nitrification are expressed in g N m^{-2}, using the mean bulk density (n=10) for calculation.

Extraction methods and chemical analyses

Each soil sample was treated as one replicate and thoroughly mixed; then 17.5 g soil was mixed with either 50 ml double distilled water (for determination of water-extractable elements), or with 50 ml 0.2 M NaCl solution (for determination of exchangeable elements). The mixtures were shaken for one hour (120 movements min^{-1}), after which the pH of the solution was measured (Radiometer type PHM 82 pH-meter). After that, the solution was centrifuged (12000 rpm, 20 min) and the supernatant was stored in polyethylene bottles at -28 °C. Al, Ca, Mg and P concentrations were measured using an ICP (type IL Plasma 200), NO_3^- and NH_4^+ concentrations were determined colorimetrically with a continuous-flow autoanalyser (Technicon AAII system) and K concentrations were quantified with flame photometry (Technicon Flame photometer IV).

In order to determine the total C and total N contents of the soil samples, $CaCO_3$ had to be removed from the soil. Hence, 10 ml 1 M HCl was added to 5 g of dry, thoroughly ground soil, and the mixture was heated to 105 °C for 12 hours. After cooling down, the soil samples were analysed for total C and total N using a CNS analyser type NA 1500 (Carlo Erban Instruments).

Statistical analyses

In order to determine differences in top-soil chemistry, net mineralisation and net nitrification between vegetation zones, analyses of variance were performed (n=10, GLM procedure, SAS 6.0). A repeated measures procedure was used in order to determine time effects and interactions between time and vegetation zone. Data on soil chemistry were log-transformed in order to fit a normal distribution.

RESULTS

Hydrology

Groundwater influence in the rooting zone differed largely between vegetation zones and years (Table 2, Figure 1). Almost every year, the groundwater reached the rooting zone (defined as the upper 10 cm of the soil) in winter and spring, dropped to about 100 cm below surface in summer and subsequently rose into the rooting zone in autumn. Yet, despite the fact that the difference in level of the soil surfaces of the two vegetations measures only 11 cm on average, the number of days that the groundwater reached the rooting zone differed considerably (Table 2). On average, groundwater seeped into the rooting zone of the species-rich heath for 121 (± 3) days per year, whereas the species-poor heath is drenched with groundwater only 46 (± 6) days per year.

Table 2 General soil characteristics. Significant differences are indicated by *:p<0.05, **: p<0.01, ***: p< 0.001. N=10

	Species-rich heath	Species-poor heath	Sign.
Soil organic matter (%)	11 ± 2	18 ± 1	**
Total C (%)	7.20 ± 0.52	4.87 ± 0.76	*
Total N (%)	0.33 ± 0.03	0.28 ± 0.04	ns
C:N ratio	22.09 ± 0.21	17.69 ± 0.24	***
Bulk density (kg m^{-3})	375 ± 24	460 ± 28	*
Groundwater raises into the rooting zone (number of days):			
1991-1995	121 ± 3	46 ± 6	***
april 1995 – april 1996	24	2	

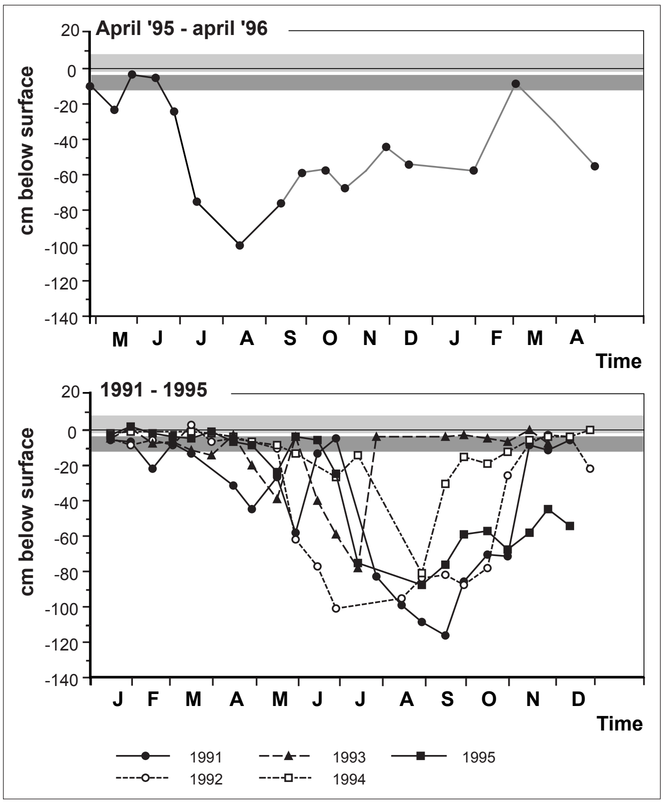


Figure 1 Groundwater fluctuations in the Breklenkampse Veld during A. the experimental period April 1995-April 1996, and B. 1991-1995. The level of the rooting zones of the species-rich and species-poor zones in relation to the soil surface at the location of the piezometer, are indicated by dark and light shading respectively.

Table 3 Chemical composition of groundwater in Breklenkamp. Concentrations in µmol l^{-1}. N=3

	Mean	SEM
pH	6.29	0.09
Ca	622.67	21.33
Na	232.09	32.83
Mg	79.83	3.54
K	34.72	10.54
NH_4^+	29.53	6.37
Zn	14.62	1.12
Fe	4.65	1.68
Mn	2.54	0.18
Al	1.53	0.40
HCO_3^-	1660	110
Cl	162.12	43.39
S	79.93	4.48
NO_3^-	14.91	10.59
P	5.24	0.43

During the experimental year, however, the autumn and winter were extremely dry in this part of the Netherlands, and the groundwater levels remained below the rooting zone of the species-poor vegetation zone. The number of days that groundwater levels reached the rooting zone of the species-rich vegetation was also severely reduced to 24 days during the experimental year (Figure 1, Table 2).

The groundwater is moderately buffered (pH=6.29; Table 3). Calcium, sodium and magnesium were the main cations, whereas bicarbonate was the dominant anion. Samples taken throughout the year did not show much variation in chemical composition, indicating a constant quality of the groundwater (Table 3).

General soil characteristics and top-soil chemistry

The soils of the two vegetation zones differ in general soil characteristics (Table 2): the soil of the species-poor heath has a higher organic matter content than the species-rich heath. Both the total organic C content and C/N-ratio were, however, lower in the species-poor zone, whereas no difference in total N content was measured between the soils of the two heath vegetations. No differences in soil moisture content of the top soil were measured between the vegetation zones during the experimental period, despite the considerable difference in groundwater influence in the rooting zone (Figure 2, Table 4).

Table 4 Anova table of the effects of vegetation zone and time, and the interaction between vegetation zone and time on soil moisture, pH, exchangeable Ca, Mg, K and NH_4^+, and water-extractable Al and Al/Ca, NO_3^-, NH_4^+, P. N=10. Significance value P<0.001:***; P<0.01:**; P<0.05:*; ns = not significant at P<0.05. The significance value is given when 0.05<P<0.10.

Variable	Vegetation zone	Time	Vegetation*time
pH	***	***	ns
Ca_{ex}	***	***	***
Mg_{ex}	***	***	ns
K_{ex}	***	***	*
Al	***	***	**
Al/Ca	***	***	***
NO_3^-	*	***	0.08
NH_4^+	*	***	ns
$NH_{4\,ex}^+$	***	***	*
P	ns	***	ns
Fe	**	***	***
Soil moisture	ns	***	***

The concentrations of minerals and nutrients differed largely between the two vegetation zones (Figure 2, Table 4). In general, factors related to soil acidity (pH, base cations, Al and Al/Ca-ratio) differed more between the zones than did factors related to nutritional status (N, P). In fact, the mineral P concentration was equal in both vegetation zones.

pH and exchangeable Ca and Mg concentrations were much higher in the lower, species-rich zone, in contrast to the exchangeable K concentration, which reached higher values in the higher zone (Figure 2). The concentrations of the acidic cation Al and the Al/Ca ratio were also lower in the lower vegetation zone. All of the factors related to the soil acidity showed a distinct response to time (Table 4). In general, they decreased throughout the experimental year, with the largest decrease during the first 100 days. This coincided with the period in which the groundwater tables fell quickly (Figures 1 and 2). However, top-soil chemistry was not affected in the same way in the two vegetation zones, as is indicated by the significant vegetation zone* time interactions (Table 4). The decrease in exchangeable base cation concentrations was much greater in the species-poor zone than in the species-rich zone (98, 67 and 45 % for Ca_{ex}, Mg_{ex} and K_{ex} respectively in the species-poor zone, and 54 and 48% for Ca_{ex} and Mg_{ex} in the species-rich zone). In the species-rich zone, K_{ex} also decreased by approximately 63% until November, but increased again to values slightly above the initial values during winter.

In April 1995, Fe concentrations in the top soil of the species-rich vegetation far exceeded those in the species-poor zone (255 and 88 µmol kg^{-1}, respectively), but they

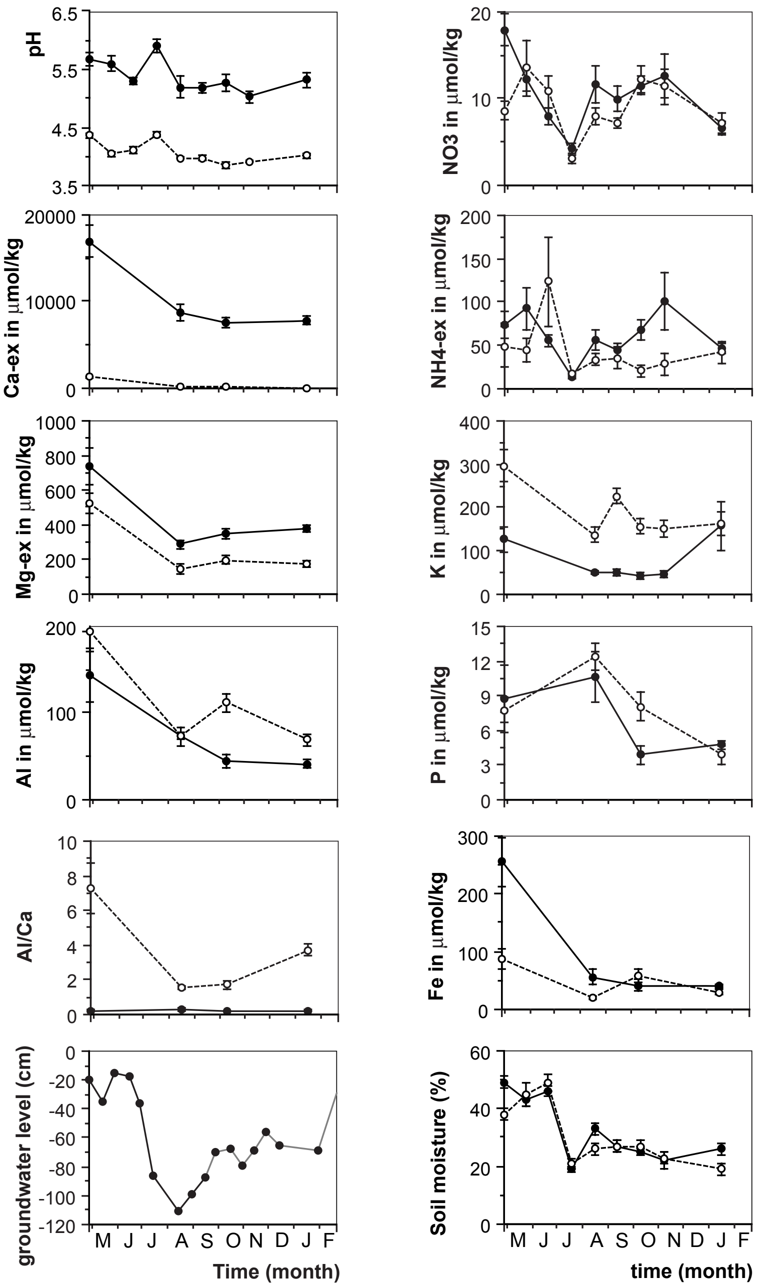


Figure 2 Time courses of pH, mineral and nutrient concentrations (mean ± SEM) in the upper soil layers of the species-poor (open circles) and species-rich (closed circles) heath vegetations. Fluctuations of groundwater (relative to the soil surface at the piezometer) and soil moisture contents are also depicted.

decreased quickly to values which were almost equal to those in the species-poor zone.

Phosphorus concentrations were very low (< 12.5 µmol kg^{-1}) and there was no difference between the two vegetation zones (Figure 2, Table 4). Top soil phosphorus concentrations decreased significantly in time, but again, no interactions between time and vegetation zone were found (Table 4).

Mineral nitrogen concentrations were also low, generally below 125 µmol kg^{-1} (NO_3^- + $NH_4^+{}_{ex}$; Figure 2, Table 4). They did, however, differ between the vegetation zones, with the species-rich heath having slightly higher NO_3^-, NH_4^+ and $NH_4^+{}_{ex}$ concentrations throughout most of the year. Only in June did the mineral nitrogen concentrations in the species-poor zone exceed those in the species-rich zone. The mineral nitrogen concentrations showed considerable variation throughout the year, yet interactions between time and vegetation zone were only significant for exchangeable NH_4^+.

Net N mineralisation and net nitrification

Net N mineralisation and nitrification occurred in both the species-rich and the species-poor heath, and on an annual basis, no differences between the zones were found. Both mineralisation and nitrification rates were low: net N mineralisation approximately 1 g N m^{-2} yr^{-1}, nitrification approximately 0.02 g N m^{-2} yr^{-1}, regardless of the vegetation zone (Figure 3). However, particularly in the species-poor zone, net N mineralisation showed a seasonal pattern ($p<0.0001$), with the highest net mineralisation in July-August, and the lowest during winter and spring. Net N mineralisation was less affected by seasonal influences in the species-rich heath, although net N mineralisation was highest in early summer (June-July; Figure 3). Despite this, cumulative net N mineralisation did not differ between vegetation zones over the year, although in May and July-August, there was a significant difference between the zones regarding mineralisation ($p<0.05$).

In the autumn and early winter, losses of nitrate were observed in both vegetation zones, indicating net immobilisation or denitrification. Nitrate losses were highest in the species-poor zone.

DISCUSSION

Ecosystem functioning

THE CALCICOLE - CALCIFUGE GRADIENT

It has been shown that large differences exist in mineral and N concentrations in the top-soil layer in the two heath vegetations. Differences in pH and base cation

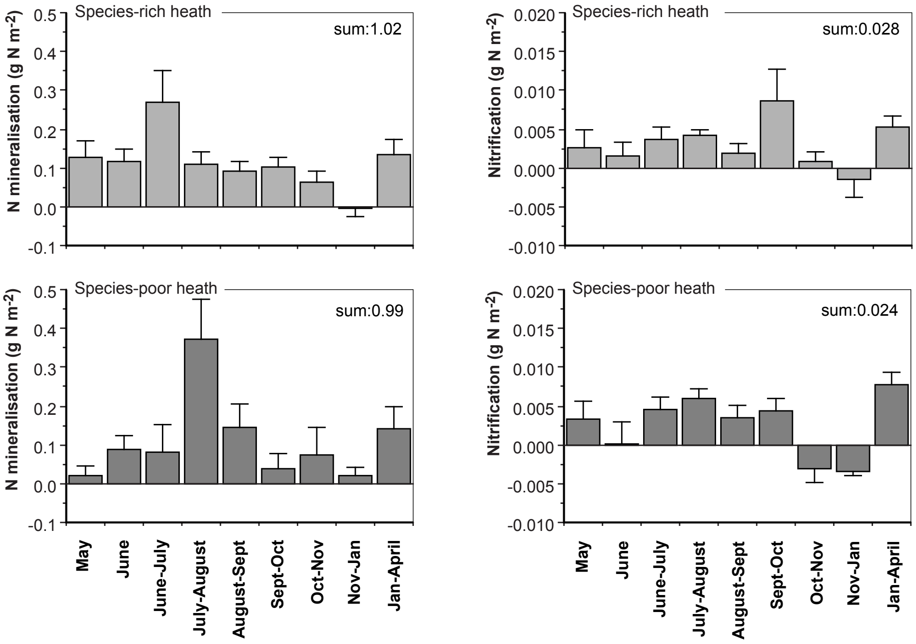


Figure 3 N mineralisation and nitrification (g N m^{-2} yr^{-1}; mean + SEM) in the two heath vegetations in the Breklenkampse Veld. Annual summations of the N mineralisation and nitrification are indicated by 'sum:'.

concentrations between the two zones were evident, as would be expected in a calcicole - calcifuge gradient. Statistical analysis on top-soil chemistry revealed that the two zones did not only differ in concentrations of minerals and nutrients, but also that there was a significantly different interaction between time and vegetation zonation (Table 4). This is an important indication that the processes defining top-soil chemistry differ between the two vegetation zones.

The distribution of the heath species correlates with a distinction in major processes that buffer soils against acidification. In acid and slightly acidic soils, three major buffering processes can be distinguished: buffering by cation exchange, by dissociation of aluminium(hydr)oxides and by weathering of silicates (Scheffer & Schachtschabel 1979). The latter mechanism is very slow, and therefore less relevant for most ecological processes.

Cation exchange processes, in which hydrogen ions are adsorbed to negatively charged soil particles in exchange for the desorption of base cations (Ca, Mg, K and Na), are the main buffering mechanism in soils with pH values between 4.5 and 6, hence in the species-rich zone (Figure 2). The desorped base cations may leach to deeper soils, thereby reducing the availability to plants. This is observed in the species-rich zone: the drop in groundwater level coincided with a drop in the concentrations of Ca_{ex}, Mg_{ex}, and Fe.

In the species-poor zone, pH values do not exceed 4.5 and base cation concentrations are low, indicating that buffering by cation exchange processes are not important in this zone. The chemical composition of the soil indicates that buffering by dissolving aluminium(hydr)oxides resulting in an increase in the Al^{3+}-concentration, is the main buffering process in the species-poor zone.

With significant differences in the number of days that the calcareous groundwater reaches the rooting zone, the differences in hydrology obviously underly the calcicole - calcifuge gradient in the heathland vegetation of the Breklenkampse Veld. Whenever groundwater levels reach the top-soil layer, the base-rich groundwater will recharge the soil complex (Table 3). The exchanged hydrogen ions will react with the bicarbonate ions, thus the buffering capacity of the soil will increase each time the groundwater contacts the soil complex. This process has been observed in fen meadows (Grootjans & Ten Klooster 1980, Jansen 2000) and wet heathlands (Jansen *et al.* 1996, Jansen *et al.* 2000), and most likely accounts for the higher pH in the species-rich heath vegetation in the Breklenkampse Veld. However, recharging of the adsorption complex has not actually been observed in the experimental period. During the extremely dry autumn and winter of 1995/1996, groundwater levels did not rise into the top-soil layers, as they did in previous winters and springs (Figure 1). Hence, base cations were not supplied to the zone, resulting in enduring low base cation concentrations. However, the biogeochemical process can be derived from the high initial values in base cations, from groundwater fluctuations over a longer period (Figure 1) and from less detailed studies on top-soil chemistry over a longer period (De Graaf *et al.* 1994). Moreover, when the groundwater fluctuations are depicted as duration lines (data not shown), they show a convex shape, indicating that the vegetations are supplied with groundwater originating from a regional base flow (Niemann 1973). The fact that the base cation concentrations in the species-rich zone do not decrease further during winter, can be explained by the fact that both plants and microbes are mostly inactive during the cold weather and hence will excrete less hydrogen ions.

Presumably, groundwater supply of base cations into the species-poor zone is not sufficient to counteract the input of hydrogen ions in this zone, as the pH of the zone remained low throughout the experimental period and base cation concentrations remained low (Figure 2). Moreover, it is likely that the groundwater that reaches the upper soil layers in the species-poor zone will be diluted with rain water, as the groundwater only reaches the upper soil layers in times of high precipitation (Jansen *et al.* 1993). Hence, base cation concentrations in the groundwater will be lower and the groundwater will add less to the recharging of the adsorption complex.

NITROGEN, N-MINERALISATION AND PHOSPHORUS

The low concentrations of ammonium and nitrate in the soil are consistent with the low N mineralisation and nitrification rates that we have observed (Figure 3). Compared to other data on N mineralisation and nitrification in heathlands, the annual N-mineralisation and nitrification are low (respectively 1 g N m^{-2} yr^{-1} and 0.02 g N m^{-2} yr^{-1} in the two zones). N mineralisation rates in Dutch heath vegetations vary from 1 - 15 g N m^{-2} yr^{-1} (Berendse *et al.* 1987, Berendse 1990, Van Vuuren *et al.* 1992). The N mineralisation rate of 1.1 g N m^{-2} yr^{-1} that has been observed in an English acidic grassland (Morecroft *et al.* 1994) is similar to the values obtained in our study.

Low N mineralisation rates in Dutch heathlands have been observed in young, 2-10 yr old wet heath vegetations (Berendse 1990). In his study, Berendse showed a clear relation between the age of heath vegetations developing after sod cutting and the N mineralisation rate, in which N mineralisation rates increased with the age of the dwarf-shrub dominated vegetation. This held especially for heath vegetations which were older than 10 years, as N mineralisation rates initially decreased after sod cutting.

The accumulation of the organic matter was also positively correlated with the age of the stand (Berendse 1990). As accumulation of organic matter seems to be an important factor for N mineralisation, it could explain the low N mineralisation in the Breklenkampse Veld, despite the fact that these vegetations are much older than ten years. Here, organic matter accumulation is low because of the frequent mowing with subsequent removal of the hay.

In general, Dutch heaths are mown less frequently than the Breklenkampse Veld (every 10 years or even less frequently; Aerts & Heil 1993 cf Van der Zande 1988), if mown at all. The frequent mowing regime in the studied area may be an important factor in the conservation of the site. During the last decades, many wet heathlands have turned into *Molinia caerulea* dominated grasslands as a result of increased N-availability (Aerts & Berendse 1988, Berendse 1990). An important factor in this secondary succession is the increased atmospheric deposition of nitrogen, which causes enhanced accumulation of organic matter followed by an increase in N mineralisation (Berendse 1990).

The quality of litter is often defined by its C/N ratio (Heal *et al.* 1997). In Dutch heathland soils, the C/N ratios generally vary between 20 and 30 (De Boer 1989, Van Vuuren *et al.* 1992, Jansen *et al.* 1996). Jansen *et al.* (1996) showed that C/N ratios in wet heathlands varied with the vegetation, with lower ratios in species-rich vegetations (C/N 20 – 25) than in dwarf-shrub dominated species-poor heathlands (C/N 30-45). In contrast, in the heathland vegetations of the Breklenkampse Veld, higher C/N-ratios were found in the top soil of the species-rich zone than in the species-poor zone (Table 2).

In a mineralisation study by Van Vuuren *et al.* (1992), the C/N ratio of the upper soil layers in an acid wet heath showed a correlation with species composition as well as with the N mineralisation rate. However, in the Breklenkampse Veld, no differences in N mineralisation rates were found between the vegetation zones. This might suggest that other factors, e.g. environmental conditions or substrate quantity, are more important than C/N ratio in determining N mineralisation. However, it is also possible that the mineralisation processes in both zones do respond to litter quality and environmental conditions, but that the outcome of all of the processes is equal for both zones. This cannot however be deducted from the results and more detailed studies will be necessary to elucidate this problem.

Nitrification, as we have measured, is the result of nitrate formation and losses by denitrification. Yet, it is highly unlikely that differences in nitrification are masked by differences in denitrification between the zones, as denitrification from heathlands with such low nitrification is highly unlikely (Van Vuuren *et al.* 1992). Hence the results give an adequate picture of nitrification in the two zones. Yet, despite the much higher pH in the species-rich zone, no differences in annual net nitrification have been observed between the two zones (Figure 3). This does not agree with the results of laboratory studies with heathland soils by Roelofs *et al.* (1985), which showed a marked decrease in nitrification with a decreasing soil pH. However, Troelstra *et al.* (1990) showed that nitrification rates in acid heaths correlated with N mineralisation rates, with the initial nitrate concentration at the start of the growing season and with the labile organic P content of the soil. Though these observations were restricted to acid, species-poor heaths, the almost equal N mineralisation rates and P-availability in the Breklenkampse Veld could explain the rather identical nitrification rates in the two studied vegetation zones.

Phosphorus concentrations did not differ between the zones (Figure 2), in contrast to other studies on the calcicole - calcifuge gradient in heaths (Houdijk *et al.* 1993b, Hayati & Proctor 1990). The concentrations are very low, but in the same order of magnitude as the phosphate concentrations in many other wet heathlands (De Graaf *et al.* 1994, Houdijk *et al.* 1993b, Jansen *et al.* 1996). In a study on P mineralisation on bogs and fens, it was shown that P mineralisation was negatively correlated with pH, thus reaching higher values in bogs than in fens (Verhoeven *et al.* 1990). Although mineralisation rates do not account solely for the actual phosphate concentrations in the soil, this observation does agree with the observations on P availability in wet heaths, in which the dwarf-shrub dominated, acidic vegetations had a higher P-availability than less acidic heath vegetations.

PLANT ADAPTATIONS

Yet, the explanation for the distribution of the plant species in relation to the calcicole - calcifuge gradient should be sought in the tolerance and adaptation mechanisms of the plant species to the environmental conditions. It is generally held that species from acid soils ($pH < 4.5$) are adapted to a number of specific conditions, e.g. high concentrations of hydrogen, aluminium, iron and manganese, and they tolerate a low availability of calcium (Hackett 1965, Gigon & Rorison 1972, Marschner 1991, Rorison 1985, Runge & Rode 1990, De Graaf *et al*. 1997, 1998). Species of less acidic habitats probably lack these special adaptations, and are, therefore, restricted to less acid soils (Gigon & Rorison 1972, Rorison 1985, De Graaf *et al*. 1997, 1998).

Furthermore, it has been shown that species from the acid soils, in which ammonium is generally far more available to plants than nitrate, have adapted to these conditions by favouring ammonium above nitrate as a mineral nitrogen source (Hackett 1965, Gigon & Rorison 1972, Rorison 1985, Falkengren-Grerup & Lakkenborg-Kristensen 1994, Troelstra *et al.* 1995). Experiments have shown that the development of acid heath species is even negatively affected when nitrate is applied as a sole or major nitrogen source (Gigon & Rorison 1972, Rorison 1985, Troelstra *et al.* 1995, De Graaf *et al.* 1998).

In contrast, the species of less acid habitats prefer nitrate or a mixture of ammonium and nitrate (Gigon & Rorison 1972, Rorison 1985, Troelstra *et al.* 1995). High ammonium concentrations, or a sole ammonium nutrition, are even toxic to *Cirsium dissectum*, a species which is characteristic of wet, slightly acidic heathlands (De Graaf *et al.* 1998). These differences in preference for nitrate or ammonium are caused by differential mechanisms for plant uptake of nitrate and ammonium.

The heath vegetations in the Breklenkampse Veld, however, do not differ much in mineral nitrogen supply (Figure 2). In both zones, nitrate as well as ammonium occured, with ammonium being the dominant mineral nitrogen form (Figure 2). Water-extractable ammonium concentrations (data not shown) remain low throughout the year, generally varying between 10 and 50 μmol l^{-1} , with a maximum of 95 μmol l^{-1}. As ammonium toxicity in heathland species is only observed at concentrations of 100 μmol l^{-1} or more (De Graaf *et al.* 1998), toxicity of ammonium is probably not important. Unbalanced uptake of nitrogen as a result of large differences in ammonium and nitrate concentrations, or due to a temporary absence of nitrate, are also not likely to occur: both ammonium and nitrate have been determined in the soil solution throughout the year. This led us to conclude that conditions concerning the mineral nitrogen nutrition are probably not distinguishing in the distribution of the heathland species in the Breklenkampse Veld.

More likely, differences in Al/Ca ratio and in aluminium concentrations in the soil solution are distinguishing in the distribution of the species. Aluminium and Al/Ca-ratio are significantly higher in the species-poor than in the species-rich zone (Figure 2). The aluminium concentrations in the species-poor zone equal or exceed the toxic levels that have been established for characteristic species of species-rich heaths (Houdijk *et al.* 1993b, De Graaf *et al.* 1997). High aluminium concentrations (150 μmol l^{-1}) have also been measured in the species-rich zone in April 1995. However, they were accompanied by high calcium concentrations, resulting in very low Al/Ca-ratios, much lower than in the species-poor zone. As the expression of aluminium toxicity is not only determined by the absolute aluminium concentration, but also by the Al/Ca-ratio (De Graaf *et al.* 1997), these differences are likely to account for the differences in the species distribution in the two vegetation zones.

Implications for the conservation of wet heathlands

Wet heathlands are declining throughout Western Europe (Aerts & Heil 1993), and especially the characteristic species of the species-rich wet heaths are becoming increasingly rare (Van Dam *et al.* 1986). Most wet heathlands are at present restricted to nature reserves. During the last decades, however, the decline of the heathlands has mainly been caused by atmospheric deposition of N and S (Roelofs 1986, Aerts & Berendse 1988, Aerts & Heil 1993), and atmospheric deposition does not stop at the borders of the reserves. In 1989, atmospheric deposition of NH_4^+ in two Dutch heathland sites varied from 2.3 to 3.0 g N m^{-2} yr^{-1}, whereas NO_x deposition varied from 0.6 – 1.3 g N m^{-2} yr^{-1} (Bobbink *et al.* 1992). Thus, input of mineral nitrogen from atmospheric deposition exceeds the mineral nitrogen generated by N mineralisation and nitrification in the studied wet heath vegetations (c. 1 g N m^{-2} yr^{-1}; Figure 3). This has also been observed for English acidic semi-natural grasslands (Morecroft *et al.* 1994).

Atmospheric deposition of nitrogen may threaten the wet heath vegetations in three ways (Bobbink *et al.* 1998): by accumulation of nitrogen resulting in enhanced availability of ammonium and nitrate, by the soil mediated effects of acidification and thirdly by an increased susceptibility to secondary stress factors.

The effects of increased availability of mineral nitrogen after nitrogen accumulation are well documented for the dwarf-shrub dominated heath vegetations (Berendse & Aerts 1984, Aerts & Heil 1993). Eventually, the dwarf-shrub dominance has been changed into monotonous stands of *Molinia caerulea*, merely as a result of the better competitive ability of the latter species at higher N availability. The effects of increased N-availability on species-rich heaths have not been studied in field experiments, but from N fertilization experiments in neutral-acidic grasslands (Mountford *et al.* 1994, Tallowin & Smith 1994) it is known that some grasses, e.g. *Holcus lanatus* and *Lolium perenne*, benefit from the increased N at the expense of forbs such as *Cirsium dissectum*, *Lychnis flos-cuculi* and *Lotus pedunculatus*. *Cirsium dissectum* and *Holcus lanatus* are species that are also found in species-rich wet heaths. However, increased nitrogen availability might also arise from changes in hydrology. Lowering of the groundwater tables has been shown to increase N mineralisation in fen meadows (Grootjans *et al.*1985), which leads to an increase in N availability.

The present study shows that with the current N deposition, mineralisation of N should be kept low in order to keep the mineral N concentration in the soil low. The results suggest that frequent mowing of the heath vegetations with removal of the hay is an adequate method to sustain low N mineralisation rates and thereby low N availability.

Secondly, high atmospheric N deposition affects plant growth by acidifying the soils. It is thought that acidification is only important in habitats where species are not adapted to acid soil conditions, hence in species-rich heathlands. This is in agreement with the results of laboratory experiments on the performance of heathland species (Van Dobben 1991, De Graaf *et al.* 1997, 1998). High nitrification rates (especially in soils with pH > 4.5) may partly account for the soil acidification (Houdijk *et al.* 1993a). However, it has been shown that nitrification in heathlands is also related to N mineralisation, by substrate limitation (Troelstra *et al.* 1989, this study) and thereby by accumulation of soil organic matter and thus indirectly by management practices (this study). Furthermore, soils may acidify through the uptake of ammonium by plants, as ammonium uptake is accompanied by the excretion of hydrogen ions (Raven & Smith 1976, De Graaf *et al.* 1998). The present study shows that a regular supply of base cations and alkalinity to the upper soil layers prevents the species-rich heath from acidifying. Yet, in many Dutch wet heathlands groundwater tables have dropped considerably during the last decades. The effects of the hydrological changes may add to the effects of atmospheric N deposition, by enhancing soil acidification, as base-rich groundwater does not, or not long enough, reach the top-soil layer anymore.

In conclusion, the calcicole - calcifuge gradient in the wet heath of the Breklenkampse Veld is presumably based on differences in hydrology. In addition, it has been shown that the low N mineralisation and nitrification rates are important features of both studied heath vegetations. As species-rich heaths require both low nutrient concentrations and moderately acidic soils, they are threatened both by atmosspheric N deposition and the lowering of the groundwater tables. It is suggested that the low N-mineralisation and nitrification rates partly account for the fact that the wet heaths of the Breklenkampse Veld have not deteriorated, whereas many other wet heathlands have. The frequent mowing with removal of hay is probably the underlying process accounting for the low N mineralisation and nitrification rates, as frequent mowing prevents the accumulation of organic matter and nitrogen.

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Chapter 3

DIFFERENTIAL EFFECTS OF AMMONIUM AND NITRATE ON THREE HEATHLAND SPECIES

WITH ROLAND BOBBINK, JAN G.M. ROELOFS AND PETER J.M. VERBEEK

DIFFERENTIAL EFFECTS OF AMMONIUM AND NITRATE ON THREE HEATHLAND SPECIES

ABSTRACT

Arnica montana and *Cirsium dissectum*, typical of species-rich heathlands and acidic grasslands, have declined rapidly in The Netherlands in recent years. Field surveys suggest that the decline is caused by soil acidification as a result of enhanced atmospheric N and S deposition.

Therefore, the survival, growth and development of these species were studied in a water culture experiment, using nutrient solutions which differed both in mineral nitrogen form and in ammonium concentration. For comparison, the performance of a third, acid tolerant species, *Calluna vulgaris*, was studied.

The results showed that both *Arnica* and *Cirsium* performed better using nitrate than when using ammonium as a sole nitrogen source, whereas ammonium toxicity became apparent when ammonium concentrations were raised above 100 µM. Ammonium toxicity was expressed by an increase in mortality of *Arnica* plants with increasing ammonium concentrations and by a reduction of biomass in *Arnica* and *Cirsium*. Furthermore, cation concentrations in both roots and shoots decreased when ammonium was supplied as a nitrogen source. In contrast, *Calluna* showed optimal development when using ammonium as a sole nitrogen source. In this species, only root biomass was negatively affected by high ammonium concentrations.

The ecological implications of these preferences are discussed in relation to soil acidification.

INTRODUCTION

Ericoid heathlands once made up a considerate part of the Dutch landscape: it has been estimated that at the beginning of the nineteenth century 800.000 ha. of heathlands were present in The Netherlands (De Smidt 1981). In general, heathlands occur on nutrient-poor, acidic soils. Slight spatial differences in soil moisture and buffering capacity, resulted in a variety of habitats with a great diversity of plant species. However, both the area and plant diversity of heathlands have declined drastically since then, mostly due to changes in land use and agricultural practice (e.g. Aerts & Heil 1993). A further deterioration in the quality of Dutch heathlands has been observed in recent decades, which has been ascribed to enhanced atmospheric inputs of acidifying and eutrophying pollutants, in particular N and S (Roelofs 1986, Van Dam *et al.* 1986, Bobbink *et al.* 1992). At present, typical heathland species such as *Arnica montana*, *Narthecium ossifragum*, *Pedicularis sylvati-*

ca, *Gentiana pneumonanthe* and *Dactylorhiza maculata* are among the species most threatened by atmospheric deposition in Western Europe (Tickle *et al.* 1995).

Atmospheric nitrogen input not only increases the availability of mineral nitrogen in the soil, it may also lead to shifts in the dominant mineral nitrogen form, from nitrate to ammonium. Ammonium concentrations may increase directly after NH_y-deposition onto soils with a low nitrification capacity, or by inhibition of nitrification by soil acidification (Van Breemen & Van Dijk 1988). This again leads to higher ammonium to nitrate ratios in the soil. Both the high nitrogen concentrations and the shift in dominant mineral nitrogen form may affect vegetaion.

Firstly, increased nitrogen availability has changed the species composition of heathlands. Dwarf-shrub communities, dominated by *Calluna vulgaris* or *Erica tetralix*, have shifted to homogenous stands of the grasses *Deschampsia flexuosa* or *Molinia caerulea* (Heil & Diemont 1983, Aerts & Heil 1993). The increased nitrogen availability is primarily caused by enhanced nitrogen deposition, which results in accumulation of nitrogen in the system. Under these circumstances, the grasses have higher competitive abilities than the dwarf shrubs and will eventually outcompete the dwarf shrubs. In N-enriched Calluna heaths, the grasses become especially dominant after the opening of the original canopy by fire, frost or heather beetle plagues (Aerts & Heil 1993).

Secondly, high nitrogen concentrations affect plant physiology and morphology: in general, shoot/root ratios increase with increasing nitrogen availability and plants with relatively larger shoots in comparison to their root system are more sensitive to desiccation (Marschner 1995). Stewart & Nilsen (1992) observed an increase in shoot/root ratio in *Drosera rotundifolia* in a nitrogen fertilisation experiment in the field. In a study with the same species, Redbo-Torstensson (1994) showed increased mortality after nitrogen fertilisation. He hypothesized that this might partly be due to desiccation after the shoot/root ratio increased as a result of the applied nitrogen.

Thirdly, growth of many plant species is influenced by the dominant mineral nitrogen form, ammonium, nitrate or a combination of both. In general, species of acidic habitats have a preference for ammonium as a nitrogen source whereas species of less acidic habitats favour nitrate or a mix of both nitrogen forms (Gigon & Rorison 1972, Falkengren-Grerup & Lakkenborg-Kristensen 1994).

Enhanced ammonium concentrations suppress nitrate uptake (Marschner *et al.* 1991). The assimilation of ammonium is more energetically favourable than nitrate, which has to be reduced to ammonium before assimilation. Ammonium, however, has to be assimilated immediately, whereas nitrate may be stored in the vacuoles before assimilation. Due to this immediate assimilation, ammonium uptake demands a high quantity of carbon skeletons and, as a consequence, sugar concentrations of the roots are reduced. In the case of excessive ammonium uptake, this may lead to a reduction in root growth and eventually in shoot growth (Salsac *et al.* 1987).

Uptake of ammonium is associated with the excretion of protons, resulting in acidification of the rhizosphere (Raven & Smith 1976). Furthermore, ammonium uptake by plants is accompanied by a reduction in the uptake of other cations such as potassium, magnesium and calcium, and an increase in anion uptake. Very high ammonium concentrations may eventually lead to the exclusion of these cations, which may lead to nutritional deficiencies (Salsac *et al.* 1987, Boxman *et al.* 1991, Pearson & Stewart 1993). Nutritional imbalances arising from ammonium deposition are known to be an important factor in the die-back of European forests (Nihlgård 1985, Van Dijk *et al.* 1989, 1990).

It is uncertain whether the decline of subordinate heathland species is caused by competition with grasses, comparable to the decline of the dwarf shrubs, or driven by a change in abiotic factors. A considerable part of this group of endangered heathland species, e.g. *Arnica montana*, *Cirsium dissectum*, *Gentiana pneumonanthe*, *Narthecium ossifragum* and *Polygala serpyllifolia*, occur on slightly less acidic soils than those where the dwarf shrubs as *Calluna* and *E. tetralix* are dominating (Hayati & Proctor 1990, Houdijk *et al.* 1993, Roelofs *et al.* 1996). It has been suggested that the decline of these endangered species is mainly caused by soil acidification (Van Dam *et al.* 1986, Fennema 1992, Dueck & Elderson 1992). A survey of deteriorated and well-developed *Arnica* stands showed that ammonium concentrations were significantly higher in the top soil of deteriorated stands, besides differences in pH and pH-related factors as the Al/Ca ratio (De Graaf *et al.* 1997, Roelofs *et al.* 1996). Van Dobben (1991) showed that pH-values as low as 4.0 did not affect growth and survival of several heathland species (including *Arnica*), indicating that proton toxicity itself is an unlikely cause for the deterioration.

Therefore, we hypothesize that as the decline of these species is correlated with soil acidification and increased ammonium concentrations, they will be negatively affected by either enhanced ammonium concentrations in the soil or by a shift in nitrogen form from nitrate to ammonium. More acid-tolerant heathland species such as the dwarf-shrubs will not, or to a lesser extent, suffer from this ammonium stress. This paper presents the results of a study on the effects of ammonium vs nitrate as a nitrogen source and on the consequences of increasing ammonium concentrations on survival, growth, developement and nutritional status of three heathland species, *Arnica*, *Cirsium* and *Calluna*.

MATERIAL AND METHODS

The species

Arnica is perennial, belonging to the Compositae. It is a characteristic species of dry heathlands and dry, nutrient-poor, acidic grasslands, with soil pHs varying from 4.5 - 6.0 (Fennema 1992, Houdijk *et al.* 1993, Roelofs *et al.* 1996). *Cirsium* is also a perennial belonging to the Compositae and occurs on wet, slightly buffered heaths and related grasslands. Soil pHs of its natural habitats are slightly higher than those of *Arnica*-stands, with a median pH value of 5.4 in the Netherlands (Roelofs *et al.*

1996). *Calluna*, a dominant dwarf shrub of dry heathlands, belongs to the Ericacae. It is found on acidic soils with pH varying between 3.5 and 5.5, with a mean value of 4.1 in The Netherlands (Houdijk *et al.* 1993).

Experimental design

Seedlings of *Cirsium* and *Arnica* were used, because mortality is highest in the seedling phase in longlived perennial species. Therefore, this phase is critical for the maintenance of populations (Fenner 1987). Seedlings were obtained by germination at room temperature of achenes, originating from natural Dutch populations, on two layers of filter paper dampered with demi-water. Due to the low growth rate of *Calluna*, rooted cuttings of this species were used. In order to obtain these, shoot cuttings of *Calluna* were collected in a Dutch nature reserve and were transferred to a nutrient solution (3 Hoagland) for 42 days in order to root.

Seedlings and cuttings were transferred to the experimental nutrient solutions when root length was approximately 2 cm. At this time, the first two leaves of *Arnica* and *Cirsium* had appeared and the cotedylons were fully unfolded; shoot length of *Calluna* was approximately 5.5 cm. A few plants of *Arnica* and *Cirsium* died during the first week after transfer, but mortality before the beginning of the measurements was considered an artifact of transfer, and so these plants were replaced.

The plants were grown in a water culture system, made up of 2 l opaque containers (referred to as 'plant containers'), in which the nutrient solution was constantly refreshed (10 l week^{-1}). A pilot experiment showed that plants developed well at this rate with nutrient solutions of 100 µM NO_3^- as a nitrogen source (see below for other nutrient concentrations). Each plant container was connected to a separate storage container, filled with nutrient solution. A continuous air flow through the solution prevented the development of anoxic conditions. Three plant containers per treatment per species were used, each with cultures of 9 (*Arnica*), 6 (*Cirsium*) or 5 (*Calluna*) individuals. This difference in numbers of planted individuals was in part to allow for different growth rates of species (*Cirsium* grows faster than the other species), and in part dictated by availability of material (*Calluna*). The whole system was placed in a climate chamber, in which temperature fluctuated during the photo period between 25 °C and 27°C (16 h, light intensity 100 µE m^{-2} s^{-1}) and between 17 °C and 20 °C during darkness. Relative humidity varied between 45 and 60%, mimicking summer growing conditions.

Five treatments were applied, differing in mineral nitrogen form and concentration. The first two provided either NH_4^+-N or NO_3^--N as sole nitrogen source (both 100 µmol l^{-1}). Three further treatments (together with the NO_3^--only treatment) provided an increasing gradient of NH_4^+ concentrations (0, 100, 500, 1000 µmol l^{-1}) at a constant NO_3^- level of 100 µmol l^{-1}.

The concentrations of other nutrients (except for P) in the media were chosen low, as to represent natural conditions (De Graaf *et al.* 1994), and had the following concentrations: 200 µM K^+ (except in the NH_4^+-only treatment, where only 100 µM K^+ was added), 100 µM Ca^{2+}, 100 µM Mg^{2+}, 500 µM SO_4^{2-}, 400 µM Cl^- (except in the sole NH_4^+-only treatment, where Cl^--concentrations were 500 µM), 0.007 µM Zn^{2+}, 0.009 µM Mn^{2+}, 0.0002 µM Cu^{2+}, 0.0008 µM H_3BO_3 , 0.008 * 10^{-3} µM Mo and 0.003 µM thiaminedichloride. Fe^{2+} was applied as Fe-EDTA, 70 µM. $(Na)_2SO_4$ was used to compensate for ionic balance, which led to Na^+-concentrations in the solutions varying from 200 to 1200 µM. Phosphate concentrations were rather high (200 µM), in order to prevent growth limitation by phosphorus. The growth media in the storage containers were sampled and checked weekly for changes in the NO_3^-- or NH_4^+-concentrations due to nitrification; no fluctuations were observed. All nutrient solutions had a pH value of 4.00, which was maintained by the biweekly addition of HCl or NaOH to the storage containers. pH fluctuations in the plant containers were not controlled as they were considered to be a treatment effect, caused by the uptake of different nitrogen forms.

During the experimental period, the number of living individuals was recorded at regular intervals. In addition, leaf colour and the morphology of the root system were studied. When plant leaves covered the whole container and competition for light became likely, *Arnica* and *Cirsium* plants were harvested (after 57 and 46 days, respectively); *Calluna* was harvested after 63 days. Dry weights of roots and shoots were determined after a drying period of 24 hours at 70 °C.

Chemical analyses

For analyses of the chemical composition of shoots and roots, dried plant material of each species per treatment was pooled per container (n=3), ground in liquid nitrogen and dried again (70 °C, 24 h). Following this, 50 mg dry material was digested in 5 ml 95-97% H_2SO_4 and 2 ml 30% H_2O_2 (Van Dijk & Roelofs 1988). P, Ca and Mg concentrations were measured using an ICP (type IL Plasma 200 for *Arnica* and *Cirsium* samples, and type Spectroflame, simultaneous emmision spectrometer for the *Calluna* samples). N concentrations were determined colorimetrically with a continous-flow autoanalyser (Technicon AAII system; Kempers & Zweers 1986) and K concentrations were quantified with flame photometry (Technicon Flame photometer IV).

Statistical and graphical analyses

Survival of plants during the experimental period was tested using a PHREG procedure (SAS 6.0). This is a regression analysis of survival data based on the Cox proportional hazards model. Since no block-effect of containers could be detected, all individuals were included in the test.

Growth of surviving plants (total plant dry weight, dry weight of roots and shoots separately and shoot/root-ratio) was analysed in two ways. Firstly, the 100 µmol l^{-1} NH_4^+ treatment was compared to the 100 µmol l^{-1} NO_3^--treatment (GLM procedure, one-way ANOVA, SAS 6.0). Secondly, a regression analysis (REG procedure, SAS 6.0) was performed between the plant parameters and ammonium concentrations (0, 100, 500, 1000 µmol l^{-1}) in the presence of nitrate (100 µmol l^{-1}). In both approaches, the dry weights were log-transformed before analysis in order to fit the requirement of a normal distribution. For presentation purposes, means were transformed back, giving a close impression of the geometrical means.

A similar approach was used for the chemical composition data of the plants; both the effect of nitrate vs. ammonium, and the effect of an increasing ammonium concentration were statistically analysed using a one-way ANOVA (GLM procedure, SAS 6.0). Tukey's studentised range test was used to identify differences between treatments.

A graphical method after Timmer and Stone (1978) was used to determine the effects of nitrogen source and ammonium concentration on the performance of the plant species. Scatterplots of element concentrations versus contents were made; in each plot five categories could be ditinguished, according to the response of the plant relative to the control. When both concentration and content of an element increased in proportion to the control, the element was considered to be less limiting. In contrast, when both concentration and content decreased in proportion to the control, increased limitation is likely. When the increase in concentration was greater than that of the content, this was seen as luxury consumption. Dilution effects are characterised by an increase in content but a decrease in concentration. Toxicity is shown by a reduction in content and an increase in concentration.

RESULTS

Survival

No differences in survival percentages were found between plants grown solely on nitrate or ammonium, for all three heathland species (Figure 1). However, a gradual decrease in survival of *Arnica* seedlings growing with increasing ammonium concentrations at constant nitrate levels was obvious. The effect of high ammonium concentration on survival of *Cirsium* and *Calluna* was not statistically significant.

Growth and development

EFFECT OF NITROGEN SOURCE

Calluna plants grew significantly better with ammonium as a sole nitrogen source than with nitrate, as expressed by a higher total plant weight and shoot weight (Fig. 2). In contrast, *Cirsium* developed markedly better on nitrate than on ammonium. Shoot dry weight of *Arnica* plants was not significantly influenced by nitrogen form, in contrast to the root dry weight (Figure 2). As in *Cirsium*, root dry weight of *Arnica* was significantly less when plants were grown with ammonium nutrition rather

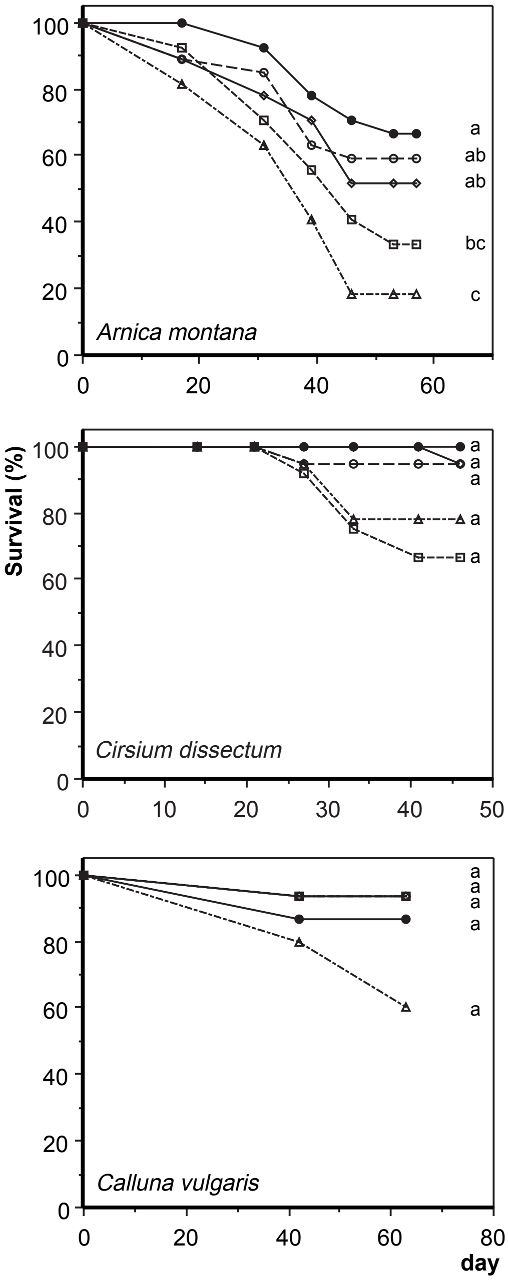

Figure 1 Survival (%) of *Arnica montana*, *Cirsium dissectum* and *Calluna vulgaris* on nutrient solutions differing in ammonium and nitrate concentrations. Significant differences between treatments are indicated for each species by different letters. Treatments: closed circles: 0 µmol $l^{-1}NH_4^+$, 100 µmol l^{-1} NO_3^-; open circles: 100 µmol l^{-1} NH_4^+, 0 µmol $l^{-1}NO_3^-$; diamants: 100 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; rectangles: 500 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; triangles: 1000 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-

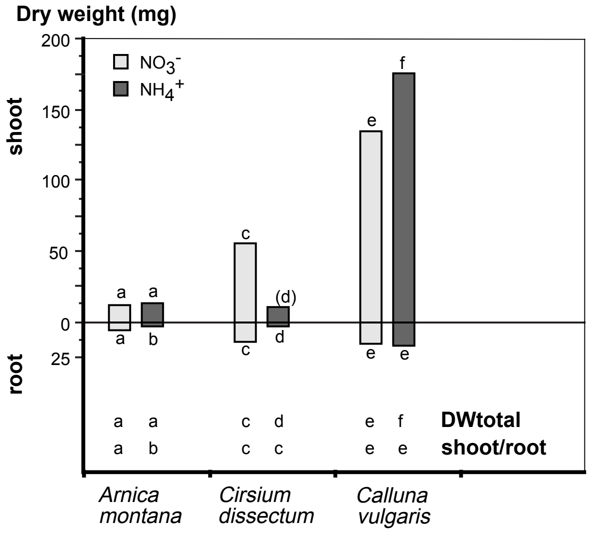

Figure 2 Geometrical means of total plant dry weight (DWtotal, mg), dry weights of shoots (DWshoot, mg) and roots (DWroot, mg) and shoot/root-ratio of *Arnica, Cirsium* and *Calluna*, grown on nitrate (100 µmol l^{-1}) or ammonium (100 µmol l^{-1}) as a sole nitrogen source. For each species, statistically significant differences ($p<0.05$, n=3) between treatments are indicated by different letters.

than with nitrate. Visual inspection of the root system of *Arnica* and *Cirsium* showed yellow-brown discolourations and poor development of the lateral roots ('stunted roots'). In contrast, roots of plants grown on nitrate were white and possessed a fully developed branched system. The roots of *Calluna* developed a fully branched root system regardless of nitrogen source.

Shoot/root ratios in *Arnica* were significantly increased with ammonium as a nitrogen source; in the other species, a tendency existed to higher shoot/root ratios in plants grown on the ammonium solution (Figure 2).

EFFECT OF INCREASING NH_4^+-CONCENTRATION

Growth of *Cirsium* was negatively affected by an increase in ammonium concentrations in the nutrient solution (Table 1). The dry weights of roots, shoots and total plants in the 1000 µM NH_4^+ treatment were less than one third of the dry weights in the 0 µM NH_4^+ treatment. Root growth was also inhibited by ammonium in *Arnica* and *Calluna*; in *Arnica* the dry weight of the roots decreased gradually from 5.77 to 1.70 mg with increasing ammonium concentrations from 0 to 1000 µM. The root dry weight of *Calluna* was not affected by 100 uM NH_4^+, but lower dry weights were observed at higher ammonium concentrations. No significant effects of ammonium on shoot or total plant weight were found for *Arnica* or *Calluna*. However, shoot dry weight of *Calluna* increased at the 100 µM NH_4^+ treatment, but decreased again at higher ammonium concentrations.

Table 1 Total plant dry weight (DWtotal, mg), dry weights of roots (DWroot, mg) and shoots (DWshoot, mg) and shoot/root-ratio of *Arnica, Cirsium* and *Calluna*, grown on nutrient solutions with increasing ammonium concentrations (0, 100, 500 and 1000 µmol l^{-1}). Mean ± SEM for shoot/root-ratio, geometrical means for DWtotal, DWshoot and DWroot. n=3, exept for 500 µmol l^{-1} treatment for *Cirsium* and the 1000 mmmol l^{-1} treatment for *Calluna*, where n=2. Sign.:significance, effect of ammonium (ns:p>0.05, *:p<0.05, **: p<0.01, ***: p<0.001)

	NH_4^+=0	**NH_4^+=100**	**NH_4^+=500**	**NH_4^+=1000**	**sign.**
Arnica montana					
DWtotal	18.64	14.69	12.39	15.84	ns
	(14.39 - 22.90)	(11.20 - 18.20)	(7.91 - 16.88)	(9.63 - 22.10)	
DWshoot	12.88	12.26	10.41	14.15	ns
	(9.00 - 16.78)	(9.17 - 15.35)	(6.74 - 14.09)	(8.66 - 19.67)	
DWroot	5.77	2.46	1.99	1.70	***
	(5.40 - 6.13)	(2.05 - 2.86)	(1.17 - 2.80)	(0.94 - 2.46)	
shoot/root	3.52 ± 0.95	5.14 ± 0.76	6.21 ± 0.91	8.43 ± 1.57	*
Cirsium dissectum					
DWtotal	69.86	30.86	45.24	20.95	*
	(52.95 - 87.03)	(25.17 - 36.58)	(20.29 - 70.81)	(13.31 - 28.66)	
DWshoot	56.15	23.44	35.44	16.57	*
	(37.29 - 75.35)	(18.70 - 28.20)	(16.17 - 55.06)	(10.36 - 22.81)	
DWroot	14.21	7.47	9.93	4.39	*
	(12.00 - 16.43)	(6.32 - 8.62)	(4.12 - 15.76)	(2.75 - 6.03)	
shoot/root	3.64 ± 1.27	3.23 ± 0.28	3.66 ± 0.17	4.14 ± 0.38	ns
Calluna vulgaris					
DWtotal	149.02	184.21	152.88	126.88	ns
	(137.25 - 160.91)	(165.94 - 2002.76)	(138.71 - 167.22)	(123.34 - 129.44)	
DWshoot	134.59	169.24	143.40	118.23	ns
	(124.13 - 145.16)	(152.44 - 186.29)	(129.47 - 157.50)	(114.31 - 122.18)	
DWroot	14.66	15.48	9.60	8.29	*
	(13.31 - 16.02)	(13.62 - 17.34)	(8.97 - 10.23)	(7.24 - 9.26)	
shoot/root	10.01 ± 0.49	13.01 ± 1.55	19.66 ± 4.15	19.96 ± 2.61	*

Visual inspection showed that lateral root development was increasingly inhibited with increasing ammonium concentrations in *Arnica* and *Cirsium*, but not in *Calluna*. In some individuals, the leaves of *Cirsium* and *Arnica* showed interveinal chlorosis, which was most obvious in the younger leaves. This indicates deficiency of magnesium (Marschner 1995). In other plants, the youngest leaves yellow discoloration in the 1000 µM NH_4^+-treatment (pointing towards calcium deficiency; Marschner 1995), whereas the leaf tips of *Cirsium* and the edges of the older leaves of *Arnica* were necrotic (symptom of potassium deficiency; Marschner 1995). In general, these signs became more pronounced with increasing ammonium concentration in the growth media. In *Calluna*, no signs of reduced vitality were observed.

The shoot/root ratio increased with increasing ammonium concentrations in *Arnica* and *Calluna*, but no effect was found on shoot/root ratios of *Cirsium*.

Nutrient concentrations of shoots and roots

EFFECT OF NITROGEN SOURCE

In all three species, nitrogen concentrations were higher when plants were grown on ammonium instead of nitrate, although this increase was not always significant in the roots (Table 2). Also, phosphorus concentrations were higher in *Cirsium* shoots when grown on ammonium, as were phosphorus concentrations in the roots and shoots of *Calluna*. In contrast, phosphorus concentrations in the roots of *Cir-*

Table 2 Nutrient concentrations (% of DW) in roots and shoots of *Arnica montana*, *Cirsium dissectum* and *Calluna vulgaris*. Plants were grown on nutrient solutions containing 100 µmol l^{-1} NO_3^- or 100 µmol l^{-1} NH_4^+ as a sole nitrogen source. N=3. bd=beneath detection limit. Different letters indicate differences in nutrient concentrations between N-sources, within species.

Shoot concentrations

	Arnica montana		*Cirsium dissectum*		*Calluna vulgaris*	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
N	3.32^a	4.14^b	3.62^a	4.70^b	1.70^a	3.32^b
P	1.58^a	1.07^a	0.83^a	1.22^b	0.29^a	0.50^b
K	10.23^a	7.26^b	4.29^a	5.26^a	0.92^a	0.29^b
Ca	0.82^a	0.55^b	1.92^a	0.92^b	0.14^a	0.08^b
Mg	0.33^a	0.35^a	0.94^a	0.53^b	0.14^a	0.11^b

Root concentrations

	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
N	1.65^a	4.55^b	3.15^a	6.29^a	2.20^a	2.33^a
P	2.02^a	1.54^a	1.39^a	1.02^b	0.21^a	0.27^b
K	8.13^a	4.05^b	1.37^a	0.31^b	1.26^a	0.47^b
Ca	0.20^a	0.12^a	0.32^a	0.22^a	0.42^a	0.35^a
Mg	0.21^a	0.17^a	0.16^a	0.12^a	0.21^a	0.22^a

sium were significantly lower in the ammonium fed plants, and a similar tendency was noted in the shoots and roots of *Arnica*.

Almost all cation concentrations were lower in both shoots and roots of the plants grown with ammonium nutrition. Concentrations of phosphorus, potassium, calcium, and magnesium were remarkably lower in the shoots of *Calluna* than in the shoots of the two other species, regardless of nitrogen source. This might partially be due to the fact that woody parts of *Calluna* were included in the analyses. However, lower phosphorus concentrations were also observed in the roots.

EFFECT OF INCREASING NH_4^+-CONCENTRATION

For all studied species, there was a weak tendency for raised nitrogen concentrations in roots and shoots with increasing ammonium concentrations in the nutrient solutions (Table 3). However, the increase was not always progressive. Phosphorus concentrations of the plants were only affected by ammonium in the roots of *Arnica*, were they decreased with increasing ammonium nutrition, and the shoots of *Cirsium*, in which the opposite was observed.

Cation concentrations were mostly affected by ammonium in shoots and roots of *Cirsium*: they decreased with increasing ammonium concentrations. A sudden decrease in cation concentrations between the treatments without ammonium and the other treatments was observed, especially for calcium and magnesium. In *Arnica*, significant decreases in cation concentrations due to increasing ammonium concentrations were only found in the calcium concentration of the shoot and the potassium concentration of the roots. A sharp decrease in cation concentration between plants grown with and without ammonium as in *Cirsium* was never observed.

Increasing ammonium concentrations in the nutrient solution had little effect on nutrient concentrations in *Calluna*, with exception of the calcium and magnesium concentrations of the shoots.

Scatterplots of nitrogen concentration versus nitrogen content of the shoots (Figure 3) indicate different responses of the species. While nitrogen in the shoots of *Arnica* and *Calluna* becomes less limiting with increases in ammonium concentrations in the nutrient solution, this increase in ammonium seem to have a toxic effect on *Cirsium*. The reduction in cation concentration caused by increased ammonium concentration in the nutrient solution, is usually in proportion to the decrease in cation content (Figure 4; data only shown for calcium). This points towards an increased limitation when ammonium is supplied. Remarkably, the increased limitation of most cations is not proportional to the applied ammonium concentration in the nutrient solution (Figure 4, Table 3).

Table 3 Nutrient concentrations of shoots and roots of *Arnica montana, Cirsium dissectum* and *Calluna vulgaris* (% of DW), grown on nutrient solutions differing in NH_4^+-concentrations. Mean values (N=3). Significant differences between ammonium treatments within species are indicated by different letters. bd= beneath detection limit.

Arnica montana

	shoot				root			
NH_4^+	0	100	500	1000	0	100	500	1000
N	3.32^{a}	4.70^{b}	4.21^{ab}	4.77^{b}	1.65^{a}	4.38^{a}	3.84^{a}	3.36^{a}
P	1.58^{a}	1.33^{a}	1.38^{a}	1.07^{a}	2.02^{a}	2.04^{a}	1.05^{b}	0.82^{b}
K	10.23^{a}	6.13^{a}	8.79^{a}	8.33^{a}	8.13^{a}	7.05^{ab}	2.97^{c}	4.71^{bc}
Ca	0.82^{a}	0.65^{ab}	0.47^{b}	0.44^{b}	0.20^{a}	0.26^{a}	0.04^{a}	0.16^{a}
Mg	0.33^{a}	0.34^{a}	0.36^{a}	0.32^{a}	0.21^{a}	0.14^{a}	0.21^{a}	bd^{a}

Cirsium dissectum

	shoot				root			
N	3.62^{a}	3.96^{a}	3.65^{a}	5.00^{a}	3.15^{a}	3.90^{a}	4.34^{ab}	6.55^{b}
P	0.83^{a}	0.99^{ab}	1.04^{b}	1.12^{b}	1.39^{a}	1.23^{a}	1.38^{a}	1.05^{a}
K	4.29^{a}	3.90^{ab}	2.93^{b}	1.49^{c}	1.37^{a}	1.49^{a}	1.65^{a}	1.39^{a}
Ca	1.92^{a}	1.13^{b}	1.11^{b}	1.19^{b}	0.32^{a}	0.22^{ab}	0.17^{b}	0.19^{ab}
Mg	0.94^{a}	0.58^{b}	0.66^{b}	0.59^{b}	0.16^{a}	0.10^{a}	0.09^{a}	0.08^{a}

Calluna vulgaris

	shoot				root			
N	1.70^{a}	2.39^{ab}	2.50^{b}	2.18^{ab}	2.20^{a}	2.38^{a}	2.40^{a}	2.40^{a}
P	0.29^{a}	0.39^{a}	0.35^{a}	0.42^{a}	0.21^{a}	0.29^{a}	0.23^{a}	0.31^{a}
K	0.92^{a}	0.77^{a}	0.93^{a}	0.90^{a}	1.26^{a}	1.35^{a}	1.42^{a}	1.30^{a}
Ca	0.14^{a}	0.10^{b}	0.09^{b}	0.09^{b}	0.42^{a}	0.33^{a}	0.40^{a}	0.38^{a}
Mg	0.14^{a}	0.10^{ab}	0.09^{b}	0.12^{ab}	0.21^{a}	0.18^{a}	0.20^{a}	0.20^{a}

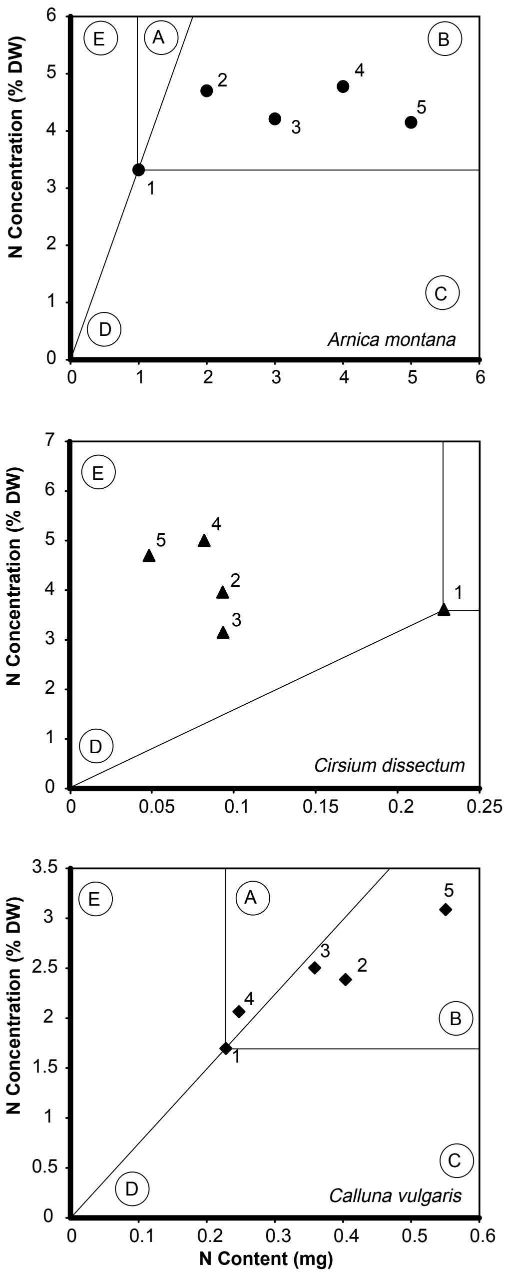

Figure 3 Concentration versus content scatterplot of nitrogen response in the shoots of *Arnica*, *Cirsium* and *Calluna*. Treatments: 1= 0 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; 2= 100 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; 3= 500 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; 4= 1000 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; 5= 100 µmol l^{-1} NH_4^+, 0 µmol l^{-1} NO_3^-. A: luxury consumption, B: decreased limitation, C: dilution, D: increased limitation, E: toxicity.

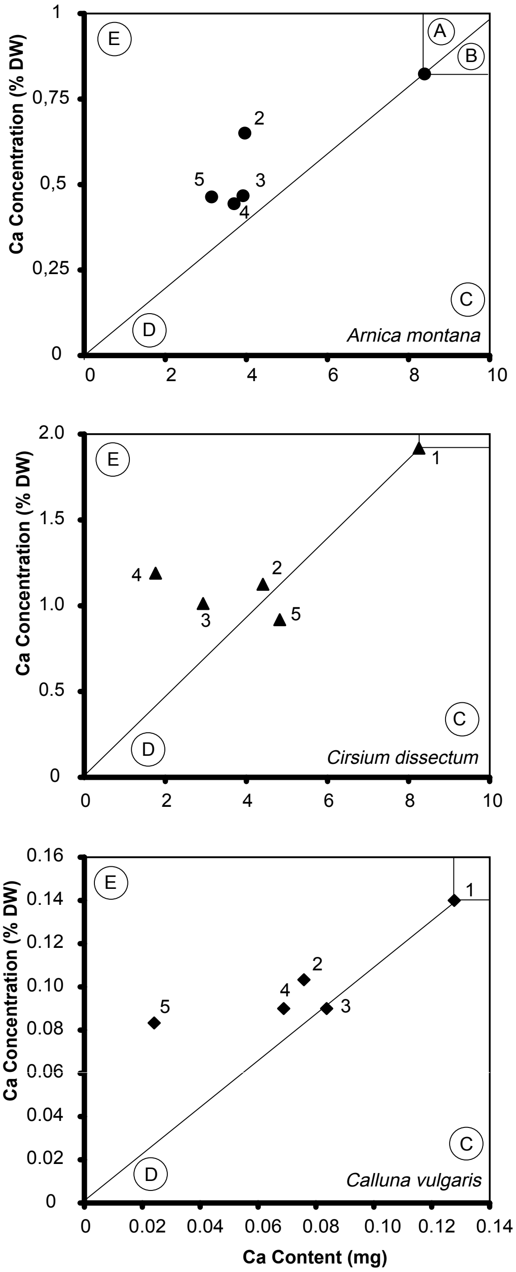

Figure 4 Concentration versus content scatterplot of calcium response in the shoots of *Arnica*, *Cirsium* and *Calluna*. Treatments: 1= 0 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; 2= 100 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; 3= 500 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; 4= 1000 µmol l^{-1} NH_4^+, 100 µmol l^{-1} NO_3^-; 5= 100 µmol l^{-1} NH_4^+, 0 µmol l^{-1} NO_3^-. A: luxury consumption, B: decreased limitation, C: dilution, D: increased limitation, E : toxicity.

DISCUSSION

The results on growth and development of the studied species emphasise their respective differences in nitrogen preference, although the survival of none of the species was influenced by ammonium or nitrate as a sole nitrogen source. While both growth and development of *Arnica* and *Cirsium* were better on nitrate, *Calluna* developed better with ammonium as the sole nitrogen source. Our results agree with earlier studies on the nitrogen preference of *Calluna* (Troelstra *et al.* 1995). In general, the results are consistent with the findings of other authors who found that species of acidic habitats, e.g. *Vaccinium corymbosum* L. , *Deschampsia flexuosa*, *Carex pilulifera, Festuca ovina, Nardus stricta* and *Juncus squarrosus* (Peterson *et al.* 1988, Falkengren-Grerup 1995, Atkinson 1985) grew better or equally well on ammonium or on a mixture of ammonium and nitrate, whereas species of weakly acidic and calcareous habitats had lower growth rates with ammonium as a sole nitrogen source (Gigon & Rorison 1972, Falkengren-Grerup & Lakkenborg-Kristensen 1994, Falkengren-Grerup 1995).

With a further increase in ammonium concentrations, the response of *Arnica* and *Cirsium* is somewhat different: whereas survival of the former species is negatively affected by higher NH_4^+-concentrations, the latter survives equally well on all treatments. However, growth and development of both shoots and roots were seriously affected in *Cirsium*, whereas only root growth decreased in *Arnica*. Both species showed the characteristic symptoms of the ammonium toxicity syndrome (Mehrer & Mohr 1989): yellow leaves and stunted roots, and these features became more obvious when ammonium concentrations increased further. Although root dry weight of *Calluna* also decreased at high ammonium concentrations (> 100 µM), stunted roots or a discolouration of the leaves were never observed. Furthermore, shoot growth was stimulated in the 100 µM NH_4^+ treatment and was only reduced in the highest ammonium treatment when compared to the lowest N-treatment. This indicates that at moderately high NH_4^+ concentrations this species benefits from the higher nitrogen availability. The relatively smaller root system might be able to obtain all nutrients required for growth (Atkinson 1985, Marschner 1995). It is, however, still unclear whether the highest ammonium concentration (1000 µmol l^{-1}) is toxic to *Calluna*.

All species had higher nitrogen concentrations in the presence of ammonium than with nitrate as a sole nitrogen source. Nitrogen concentrations increased slightly with increasing ammonium concentrations. This pattern has often been observed in studies with other species (e.g. Falkengren-Grerup 1995, Troelstra *et al.* 1995). However, species responded differently to the increased plant nitrogen concentrations: whereas the scatterplots (Figure 3) indicated that the increased nitrogen concentrations in both *Arnica* and *Calluna* could be seen as a decrease in N-limitation, in *Cirsium* the increased N had reached values that might be toxic. Thus the increased uptake of nitrogen by *Cirsium* caused, at least partly, the growth reduction. Enhanced ammonium uptake can result in carbon limitation: as ammonium which has been taken up, needs to be assimilated immediately, there is a large demand for carbon skeletons (Salsac *et al.* 1987). In fact, carbon may become limiting for growth.

Reduced uptake of cations or even cation excretion is an often observed phenomenon which accompanies ammonium uptake (Salsac *et al.* 1987, Boxman *et al.* 1991, Pearson & Stewart 1993). In this experiment, cation concentrations tended to decrease in the presence of ammonium, causing increased cation limitation (Figure 4). This is in agreement with earlier findings for *Arnica* and *Calluna* (Jenelten & Feller 1992, Troelstra *et al.* 1995). In *Calluna* the reduced cation concentrations do not correlate with reduced growth or symptoms of deficiencies. Presumably, the lower magnesium, calcium and potassium concentrations are not limiting for growth, although nutrient concentrations in *Calluna* are lower than in *Cirsium* and *Arnica*. This might be due to the fact that the woody tissues of *Calluna* were included in the analyses, thus lowering the nutrient concentration when expressed as percentage of dry weight. Differences in cation concentrations between *Calluna* and the other species are less pronounced when they are expressed as percentage of nitrogen concentration (data not shown). Moreover, plants from acid soils often have lower cation concentrations than plants from less acidic habitats (Allen 1989).

In contrast, *Arnica* and *Cirsium* leaves showed symptoms of magnesium, calcium and potassium deficiencies. These became more obvious with increasing ammonium concentrations and correlate well with the observed reductions in shoot concentrations of these cations in both species. Therefore, it is likely that enhanced nitrogen uptake and reduced cation uptake account for at least a part of the growth reduction under high ammonium concentrations in *Arnica* and *Cirsium*. Pegtel (1994) also suggested the existence of potassium-ammonium antagonism in *Arnica*, leading to nutrient imbalances and, as a consequence, reduced growth.

Ecological implications

Changes in the nutrient balance in the plant caused by increased ammonium deposition, may result in deficiencies of cations, especially magnesium and potassium. Such deficiencies are known to play an important role in the deterioration of tree species in Western European forests (Van Dijk *et al.* 1989, Van Dijk *et al.* 1990). Corresponding to our results in heathlands, the sensitivity of understorey species of moderately acidic soils to ammonium is also an effect of nutrient imbalances; species of very acidic soils are less affected (Falkengren-Grerup 1995). Uptake of ammonium is often accompanied by the excretion of protons (Raven & Smith 1976, Salsac *et al.* 1987), leading to acidification of the rhizosphere. As a result, leaching of cations may occur, thus further complicating cation uptake (Ulrich 1983).

Apart from the changes in mineral nitrogen forms, plants may also suffer from toxicity of protons, aluminium and manganese which increase to toxic levels after soil acidification. Furthermore, the solubility of phosphorus and molybdenum in soils is decreased after acidification, thus complicating plant ion uptake (Rorison 1986, Runge & Rode 1991, Marschner 1991). Little evidence exists for direct H^+ toxicity in

Arnica (Van Dobben 1991), but the importance of aluminium toxicity for *Arnica* and *Cirsium* has been shown in nutrient solution experiments (De Graaf *et al.* 1997), in pot experiments (Heijne 1995) and in correlative field studies with *Arnica* (Fennema 1992). In contrast, aluminium had no detrimental effect on *Calluna* (De Graaf *et al.* 1997), again indicating that this species is more tolerant to very acidic soil conditions than *Arnica* and *Cirsium*.

Most studies on toxicity are carried out using nutrient solutions. Although we have used rather low level nutrient solutions in order to reflect the nutrient-poor conditions of heathlands soils, it is impossible to explain the decline of species under natural conditions by the sole use of nutrient solution experiments. Ecologically important factors such as mycorrhizal infection are absent in nutrient solution experiments, but are important in nutrient acquisition from nutrient-poor soils (Chapin 1980). In a pot experiment with *Calluna*, mycorrhizal infection was reduced at higher nitrogen concentrations and a tendency also existed for lower infection rates with ammonium as a sole nitrogen source (Mickel *et al.* 1991). Yet, Caporn and coworkers (1995) found no negative effects of nitrogen fertilisation on the mycorrhizal infection of *Calluna* during a four-year field experiment, when nitrogen was supplied as NH_4NO_3. Similarly, controversies exist regarding the sensitivity of *Arnica* infected with mycorrhizas to ammonium. In a two-year field experiment, both growth and mycorrhizal infection of *Arnica* were reduced after two years of fertilisation with 90 kg ha^{-1} ammonium sulphate, indicating the potential toxicity of ammonium (Heijne *et al.* 1992). However, in a short-term pot experiment, they did not find any negative effects of ammonium treatment on *Arnica* (Heijne *et al.* 1994). Nevertheless, in a correlative field study on *Arnica* populations, ammonium was an important factor in explaining the decline of those populations (Roelofs *et al.* 1996), which is in agreement with the results of our experiment. In the same study, *Calluna* populations were found on soils with even higher ammonium concentrations than the declining *Arnica* populations, again supporting the result of this study.

Therefore, we conclude that a shift in nitrogen nutrition from nitrate to ammonium and increased ammonium concentrations are detrimental to *Arnica* and, to a lesser extent, to *Cirsium*. *Calluna* is scarcely affected. The combination of our results with those of field studies (Fennema 1992, Heijne *et al.* 1994, Roelofs *et al.* 1996) clearly indicates that ammonium toxicity in combination with soil acidification, plays an important role in the recent decline of the threatened heathland species *Arnica* and *Cirsium* in The Netherlands.

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CHAPTER 4

ALUMINIUM TOXICITY AND TOLERANCE IN THREE HEATHLAND SPECIES

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ALUMINIUM TOXICITY AND TOLERANCE IN THREE HEATHLAND SPECIES

ABSTRACT

Arnica montana and *Cirsium dissectum* are characteristic species of species-rich heathlands and adjacent grasslands, which declined during the last decades in the Netherlands. It has been shown in a recent field survey that the decline of *A. montana* and *C. dissectum* might be caused by soil acidification. *Calluna vulgaris* is not susceptible to soil acidification. It was hypothesized that increased aluminium concentrations in the soil as a result of acidifying atmospheric inputs caused the decline of *A. montana* and *C. dissectum* whereas *C. vulgaris* would not be sensitive to enhanced aluminium concentrations. We studied the effects of different Al:Ca-ratios and of Al concentrations on the development of *A. montana*, *C. dissectum* and *C. vulgaris* in nutrient solution experiments. All three species showed aluminium accumulation in the shoots related with increased aluminium concentrations in the nutrient solutions. This accumulation was correlated with a reduction in growth when plants were cultured at high Al concentrations (200-500 µmol l^{-1}), in both *A. montana* and *C. dissectum*. In addition, indications of Al toxicity were observed in these plant species, e.g. poor root development, yellowish leaves and reduced contents of Mg and P in the plants. *C. vulgaris* did not show reduced growth or poor plant development due to high Al concentrations. The negative effects of aluminium in *A. montana* and *C. dissectum* were partly counterbalanced when plants were grown on the same Al concentrations but with increased Ca concentrations, resulting in lower Al:Ca-ratios. No effects of enhanced calcium concentrations on *C. vulgaris* have been observed.

INTRODUCTION

In Western Europe, soil acidification and eutrophication occur as a result of atmospheric deposition of SO_x, NO_y, and NH_x. Both are a major threat to biodiversity in many (semi-) natural ecosystems (Bobbink *et al.* 1992). Dwarf-shrub and grassland communities occur at acidic or at slightly buffered soils in the Western-European heathland landscape, and are sensitive to both acidification and eutrophication.

As a result of enhanced nitrogen (N) inputs, the dwarf-shrub dominated communities on acidic soils have been transformed into swards dominated by grasses (Heil & Diemont 1983, Roelofs 1986, Aerts & Berendse 1988). Apart from this transition, a drastic decline in species, such as *Arnica montana* L. and *Cirsium dissectum* (L.) Hill, has been observed in Dutch heathland and adjacent grassland communities at slightly buffered soils. It has been suggested that this decline was caused by acidification as a result of SO_x deposition (Van Dam *et al.* 1986, Houdijk *et al.* 1993, De

Graaf *et al.* 1994). Field studies have demonstrated that these threatened plants are characteristic of soils in the cation exchange buffer range, with moderate pHs (4.5-6.0), whereas the dwarf shrubs (*Calluna vulgaris* (L.) Hull and *Erica tetralix* L.) mainly grow on acidic soils in the aluminium buffer range (Hayati & Proctor 1990, Houdijk *et al.* 1993). In a field survey it was shown that *A. montana*, one of the endangered species, recently disappeared from sites which have nowadays soil pH values below 4.5 and with raised aluminium (Al) concentrations (Fennema 1992).

Al toxicity after soil acidification is a widely observed phenomenon and it is generally assumed that Al^{3+} is the phytotoxic form (*Foy et al.* 1978, Ryan *et al.* 1994). The detrimental effects of high Al^{3+} concentrations have been shown particularly in forest ecosystems (e.g. Ulrich 1983; Boxman *et al.* 1991, Andersson & Brunet 1993). The effects of Al^{3+} can be diminished by high concentrations of divalent base cations, such as calcium (Ca) and magnesium (Mg) (Korcak 1990, Ryan *et al.* 1994).

We hypothesize that increased soil Al concentrations after acidification are the major cause of the decline of the threatened plant species from these slightly buffered communities. In the present study, an analysis is given of the growth response to increasing Al concentrations and to different Al:Ca ratios in a nutrient-solution experiment. The shoot and root biomass of two seriously threatened forbs (*A. montana* from dry and *C. dissectum* from wet conditions) have been studied, in contrast to those of the characteristic dwarf shrub *C. vulgaris*. At the end of the experiment, nutrient contents of the plant material were determined and the results are discussed.

MATERIAL AND METHODS

Experiments

Achenes of *A. montana* and *C. dissectum* were collected from a natural population in Dutch nature reserves (*A. montana*: Schaopedobbe, 52°57' N, 6°15' E; *C. dissectum*: 52°21' N, 7°04' E). Achenes were stored under dry and dark conditions at room temperature until the start of the experiment. Achenes were germinated in petri-dishes, on filter paper wetted with demineralised water at room temperature.

Because of the very low growth rate of *Calluna vulgaris,* shoot cuttings were used in stead of seedlings. These were collected in August 1993 from a natural heathland population (Schaopedobbe, 52°57' N, 6°15' E) and grown on nutrient solution (without Al; see below) for 63 days in order to root. The rooted cuttings of *C. vulgaris* (shoot length aproximately 6 cm; root length aproximately 2 cm) were transferred to opaque containers (2 l), as were the seedlings of *A. montana* and *C. dissectum* (root length aproximately 2 cm). The nutrient solution of each container was continuously refreshed, using 10 l medium per week. Aerobic conditions were maintained by a continuous flow of air through the containers. Plants were grown in a climate chamber at a day/night (14/10 h) temperature of 28/16 ˚C, a light intensity of approximately 100 µE $m^{-2}s^{-1}$ and with a relative humidity of 50-65%. For all species, three replicates per treatment were included, except for the 50/100 Al/Ca-treatment for *A.montana*, where n=6. This is due to the fact that the experiment with *A.montana* was performed in two groups, in both of which the 50/100 Al/Ca treatment was included. No significant differences between the 50/100 Al/Ca treatments of both groups were found in either parameter that has been tested.

Seven Al and Ca treatments were supplied (Table 1) with Al varying from 0 to 500 µmol l^{-1} and Ca from 100 to 5000 µmol l^{-1}. Treatments with low calcium concentrations were corrected for ionic strength and chloride concentration by the addition of NaCl (Table 1). Other nutrient were added in the following concentrations: 100 µM KNO_3, 100 µM $MgCl_2.6H_2O$, 100 µM $Na_2HPO_4.2H_2O$, 100 µM KH_2PO_4, 70 µM Fe-EDTA, 0.7 µM $ZnSO_4.7H_2O$, 0.8 µM $MnCl_2.4H_2O$, 0.2 µM $CuSO_4$, 0.008 µM $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.8 µM H_3BO_3 and 0.3 µM thiamin hydrochloride. In experiments with *C. vulgaris*, 100 µM $(NH_4)_2SO_4$ was added in addition, because of the preference of *C. vulgaris* for NH_4 as a N source (Houdijk & Roelofs 1993). For compensation of sulphate, only 450 µM Na_2SO_4 was added to the nutrient solutions in the *Calluna*-experiments, whereas the nutrient solutions in the experiments with *A. montana* and *C. dissectum* contained 500 µM $Na_2SO_{4.}$ The media were adjusted twice a week at pH=4.00 by HCl, in order to remain Al^{3+} in the ionic state. Never was any precipitate found in the storage or growth containers. In the plant containers, pH was allowed to fluctuate as result of the treatment; however, in the aluminium-containing treatments, pH generally remained below 4.20. In the treatments were no Al was added, pH increased to a mean value of 4.32.

The following numbers of plants were grown per container: *A. montana: 9*; *C. dissectum*: 6; *C. vulgaris:* 5. *C. dissectum* and *A. montana* were harvested after respectively 28 and 63 days, when competition for light became obvious. Because of its low growth rate, *C. vulgaris* plants were harvested after 95 days. At harvest, shoots and roots were separated and the dry weight of the shoots and roots was measured after drying at 70 °C for 24 h. The shoots were pooled per container for nutrient analyses as were roots, whenever enough plant material was available. Both were ground

Table 1 Aluminium and calcium concentrations in the nutrient solutions (in µmol l^{-1}). Aluminium was added as $AlCl_3$, calcium as $CaCl_2.2H_2O$. In addition, the NaCl-concentrations that were added in order to correct for ionic strength are given.

Medium	Al	Ca	NaCl
A	0	100	1500
B	50	100	600
C	100	100	300
D	200	100	150
E	500	100	0
F	100	1000	0
G	500	5000	0

with liquid N_2 and dried again (70 ˚C, 24 h). The plant material was digested in 5 ml concentrated H_2SO_4 and 2 ml 30% H_2O_2 (Van Dijk & Roelofs 1988). The concentrations of Al, Ca, Mg and phosphorus (P) were determined using an ICP (type IL Plasma 200). N contents were measured colorimetrically with a continuous flow autoanalyser (Technicon AAII system). Due to equipment failure, we were not able to analyse all *A. montana* plants for N.

Statistical analysis

Data were statistically analysed using a GLM procedure after testing for normality; multiple comparisons among pairs of means were made using the Tukey's studentized range test. Plant dry weight was log transformed before testing, in order to fit a normal distribution. All statistical analyses were performed using SAS 6.0.

RESULTS

Shoot and root biomass

The dry weight of shoot and roots of *A. montana* decreased gradually with increasing Al concentration and constant Ca concentration (100 µmol l^{-1}) in the nutrient solutions (Figure 1). The shoot dry weights were significantly lower in the 200 and 500 µmol Al l^{-1} treatments, whereas root biomass was only significantly lower at 500 µmol Al l^{-1}, compared with the control treatment (without Al). The shoot and root biomass of *C. dissectum* increased after the addition of 50 µmol Al l^{-1} in the nutrient solution, compared with the control treatment. At higher Al concentrations (with 100 µmol Ca l^{-1}), the shoot and root biomass decreased, however, considerably. Compared with the 50 µmol l^{-1} Al treatment, the root dry weights were significantly lower when 200 µmol l^{-1} Al or more was added to the nutrient solution. The shoot dry weight of this species decreased only significantly in the 500 µmol Al l^{-1} treatment.

The negative effects of Al on the dry weights of *A. montana* and *C. dissectum* were, although not significantly, diminished when Ca concentrations in the solutions were raised to 1000 or 5000 µmol l^{-1}, compared with the treatment with the same Al concentration but low Ca concentration.

Plant growth of the *C. vulgaris* cuttings occurred during the experimental period: in all treatments new branches and leaves were formed. The dry weights of the shoots and roots of *C. vulgaris* were, however, not affected by the different aluminium or calcium treatments (Figure 1).

The habit of *A. montana* and *C. dissectum* was also affected by increased Al concentrations; the leaves became yellow and the root development stunted. These symptoms of reduced vitality were observed in *A. montana* in solutions with 50 µmol

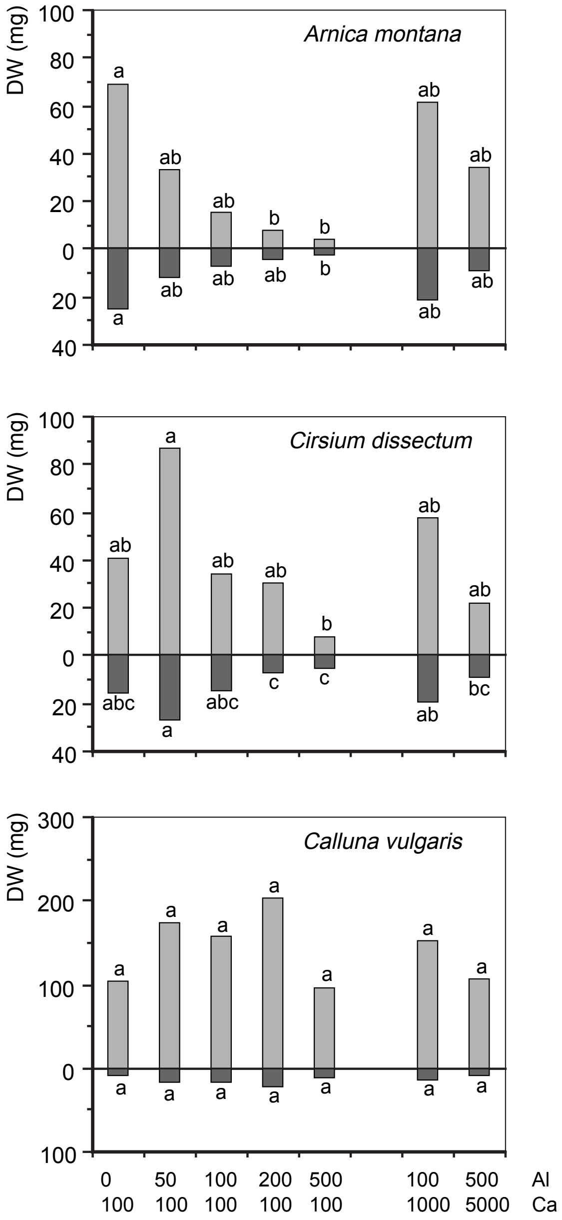

Figure 1 Mean root (dark) and shoot (light) dry weight of *A. montana*, *C. dissectum* and *C. vulgaris* treated with different concentrations of Al (0, 50, 100, 200 or 500 µmol l^{-1}) and Ca (100, 1000 or 5000 µmol l^{-1}). Significant differences within species are indicated by different letters ($p<0.05$; N=3). Significant differences in total plant dry weight follow differences in shoot dry weight for *A. montana* and *C. vulgaris*; for *C. dissectum* total dry weight was higher of plants grown on 50 µmol l^{-1} Al than dry weight of plants grown on 200 or 500 µmol l^{-1}, regardless of Ca concentration.

Al l^{-1} or more, and in *C. dissectum* in solutions with 100 µmol Al l^{-1} or more. Plants of both species which were grown on a nutrient solution with 100 µmol Al l^{-1} and 1000 µmol Ca l^{-1}, did not show the symptoms of reduced vitality; when 500 µmol Al and 5000 µmol Ca l^{-1} was applied, plants were again less vital. The just-described symptoms of reduced vitality were not observed in the plants of *C. vulgaris*, although the root system of this species was best developed in nutrient solutions with 100 or 200 µmol Al l^{-1} or more, and low Ca concentrations.

Nutrient concentrations in the shoots and roots

The nutrient concentrations in the roots of the three investigated heathland species were hardly influenced by the Al treatments, but differences in the nutrient concentrations of the shoots were observed (Table 2). In all three species Al accumulated in the shoots when the Al concentrations in the nutrient solution increased. This increase was largest in *A. montana*-shoots, followed by *C. dissectum* and *C. vulgaris* respectively. Shoot Al concentrations of *A. montana* and *C. vulgaris* were not reduced by addition of 1000 or 5000 µmol Ca l^{-1} to the nutrient solution. Addition of 5000 µmol Ca l^{-1} seemed to reduce the Al concentrations in the shoot material of *C. dissectum*, although the reduction was not significant. All three species showed higher Ca concentrations in the shoots when the plants were grown on nutrient solutions with high Ca concentrations, but no effects of increased Al supply on shoot Ca concentrations were observed. Mg concentrations in the shoot material of *C. dissectum* were, not significantly, reduced in the 500 µmol Al l^{-1} treatment. In the 100:1000 and 500:5000 Al:Ca treatments, the Mg contents in the shoots were lower than in treatments with similar Al concentrations and 100 µmol Ca l^{-1}. Mg concentrations in the shoots of *A. montana* and *C. vulgaris* were not affected by the different treatments (Table 2).

P concentrations of the roots of the three investigated species have not been influenced by any of the treatments. The P concentrations in the shoots of *C. vulgaris* were neither affected by the Al or Ca addition. The P concentrations in the shoots of *A. montana*, which were grown at 500 µmol Al l^{-1}, decreased significantly compared with the control treatment and almost independent of the applied Ca concentrations. In *C. dissectum* shoots P concentrations were, however, significantly higher in the 200 µmol Al l^{-1} treatment, compared with plants which had been supplied with 50 or 500 µmol l^{-1} Al. No effects of Al treatments on N concentrations in shoots or roots of the three investigated species were found. The Ca addition of 500 µmol or 5000 µmol l^{-1} significantly decreased the N concentrations in the leaves of *A. montana* (Table 2), but due to equipment failure we were unable to determine N concentrations of the shoots in all treatments.

Table 2 Mean nutrient concentration in leaf and root of *A. montana*, *C. dissectum* and *C. vulgaris* (in µmol g^{-1}). Significant differences between treatments are indicated by different letters ($p<0.05$). n=number of replicates, *=not determined.

	Al=0 Ca=100	Al=50 Ca=100	Al=100 Ca=100	Al=200 Ca=100	Al=500 Ca=100	Al=100 Ca=1000	Al=500 Ca=5000
***A. montana*, shoot**							
Al	3.7 a	56 ab	96 ab	148 ab	234 b	75 ab	207 ab
Ca	125 a	109 a	84 a	72 a	83 a	463 b	775 c
Mg	121 a	66 a	96 a	83 a	75 a	74 a	204 a
P	621 acd	665 a	672 ac	493 ace	231 be	462 ace	291 de
N	*	1786 a	*	*	*	1759 a	1231 b
n	3	6	3	3	3	3	2
***C. dissectum*, shoot**							
Al	n.d. a	n.d. a	24 ac	181 b	112 bcd	25 ad	23 ad
Ca	239 a	227 a	237 a	295 a	123 a	661 b	970 b
Mg	212 ab	203 ab	204 ab	290 b	88 ac	80 ac	17 c
P	178 ab	154 a	206 ab	379 b	136 a	195 ab	70 a
N	937 a	711 a	1409 a	736 a	442 a	589 a	223 a
n	2	3	2	3	3	3	3
***C. vulgaris*, shoot**							
Al	6 a	26 ab	28 ab	43 ab	84 b	24 ab	46 ab
Ca	70 ab	67 a	84 ab	63 a	63 a	243 ab	293 b
Mg	55 a	67 a	79 a	70 a	62 a	67 a	48 a
P	99 a	142 a	137 a	128 a	117 a	115 a	109 a
N	973 a	1149 a	1234 a	1272 a	1216 a	1122 a	1391 a
n	3	3	2	3	3	3	2
***A. montana*, root**							
Al	90 a	218 a	239 a	224 a	273 a	319 a	223 a
Ca	343 a	94 a	66 a	111 a	147 a	108 a	21 a
Mg	124 a	214 a	77 a	99 a	162 a	318 a	214 a
P	709 a	924 a	758 a	778 a	945 a	636 a	218 a
N	*	1532 a	*	*	*	1429 ab	806 b
n	3	6	3	3	3	3	1
***C. dissectum*, root**							
Al	n.d. a	60 a	163 a	189 a	144 a	121 a	81 a
Ca	65 a	104 a	32 a	23 a	36 a	58 a	58 a
Mg	31 a	47 a	33 a	24 a	11 a	34 a	0 a
P	165 a	268 a	499 a	472 a	182 a	452 a	187 a
N	493 a	610 a	536 a	339 a	55 a	475 a	105 a
n	2	1	2	3	3	3	3

Table 2 Continued

	Al=0 Ca=100	Al=50 Ca=100	Al=100 Ca=100	Al=200 Ca=100	Al=500 Ca=100	Al=100 Ca=1000	Al=500 Ca=5000
C. vulgaris, root							
Al	15 a	319 ab	263 ab	256 ab	412 b	272 ab	157 ab
Ca	11 a	11 a	40 a	21 a	41 a	28 a	20 a
Mg	60 a	24 a	72 a	52 a	63 a	73 a	34 a
P	189 a	308 a	374 a	406 a	521 a	438 a	225 a
N	1965 a	1106 a	1752 a	1759 a	2396 a	1748 a	1129 a
n	2	2	2	3	3	2	1

DISCUSSION

In many studies the toxicity of Al for plant species has been investigated on water culture. In order to relate the results to the field situation, it is essential that the nutrient solutions which are used resemble the soil solution as best as possible (Andersson & Brunet 1993, Sverdrup & Warfvinge 1993, Falkengren-Grerup 1994). In heathland ecosystems, nutrient concentrations of the soil are very low. The nutrient concentrations that we have used in this experiment are in good agreement with field measurements, except for P and K (data not shown). These were somewhat higher in the nutrient solution than in the field (Table 2, Matzner & Ulrich 1980, Hayati & Proctor 1990, Houdijk *et al.* 1993, De Graaf *et al.* 1994).

The growth of *A. montana* and *C. dissectum* was negatively affected by Al concentrations of 100 µmol l^{-1} and more, whereas growth of the dwarf-shrub *C. vulgaris* was not affected at all. Symptoms of reduced vitality, e.g. stunted root growth and changes in chemical plant composition, have also been observed with increasing Al concentrations for *A. montana* and *C. dissectum*. In *A. montana*, the most Al-sensitive species, root development was already influenced at 50 µmol Al l^{-1}. Stimulation of plant growth by low Al concentrations, as in *C. dissectum* in this experiment, has been observed for more species (Foy 1978). Hackett (1965) showed that growth of *Deschampsia flexuosa*, a characteristic grass species of acidic habitats, is also stimulated by low Al concentrations.

Our results concerning the effects of Al on *A. montana* do not agree with those found by Pegtel (1987) and Kroeze *et al.* (1989). They concluded that the growth of *A. montana* on water culture was not influenced by Al, not even at Al concentrations of almost 3000 µmol l^{-1}. However, already at intermediate Al concentrations Pegtel (1987) observed reduced root elongation, a reduction in fine-branching of the roots, yellowish-green leaves and the development of necrotic spots on the leaves. This indicates that plants are affected by the treatment; particularly the mentioned root morphology is characteristic of Al toxicity (Foy *et al.* 1978). The difference in the sensitiveness of *A. montana* to Al is most likely caused by the very high nutrient concentrations (especially N: 4000 µmol l^{-1} and K: 2330 µmol l^{-1}) in the media used by Pegtel (1987) and Kroeze *et al.* (1989) and the lower, more realistic concentrations used in this experiment (N: 100 µmol l^{-1}, K: 600 µmol l^{-1}). The high nutrient concentrations in solutions might enable sufficient nutrient uptake by plants, even when root vitality and nutrient uptake capacity are reduced by Al (Foy *et al.* 1978).

Chemical composition of plants differs between roots and shoots and between species and can be influenced by environmental stresses. A commonly observed phenomenon of high Al concentrations in soil or nutrient solutions is the accumulation of Al in the plant (Foy et al. 1978). In this study, Al concentrations in all three heathland species clearly increased with increasing Al concentrations in the nutrient solution (Table 2). This increase in Al concentrations in the shoots correlated with the decrease in plant biomass of *A. montana* and partly with the decrease in biomass of *C. dissectum*. Growth was not reduced in *C. vulgaris*. This species thus seems to resist high concentrations of Al in the shoot, whereas the other investigated species are affected by these Al concentrations. High Al concentrations in nutrient solutions are known to influence the uptake of minerals; particularly the uptake of the divalent cations Ca and Mg is often disturbed by Al (Foy *et al.* 1978, for a recent review see Delhaize & Ryan 1995). However, none of the three heathland species showed significant decreases in Ca concentrations in roots or shoots, while the Mg concentration was only lowered in the highest Al treatment in the shoots of *C. dissectum* (Table 2). This strongly suggests that the detrimental effects of Al in this study are not primarily caused by reduced cation uptake.

N and P are the main growth limiting factors in nutrient-poor habitats. Al significantly reduced plant P concentrations in the two species sensitive to Al (Table 2). P deficiencies sometimes appear as a result of Al toxicity (Foy 1978). It is, however, unlikely that the observed effects of Al on plant growth in this experiment are caused by P deficiency, because even in the highest Al treatment P concentrations in the plant are considerably higher than those of natural populations (Hayati & Proctor 1990, Pegtel 1994).

Negative effects of Al on growth and plant vitality were partly ameliorated in *A. montana* and, to a lesser degree, *C. dissectum* by high Ca concentrations. The beneficial effects of Ca on Al toxicity in plants have been recognized for a long time, although the mechanism by which Ca reduces Al toxicity is not yet fully understood (Rengel 1992, Kinraide *et al.* 1994, Ryan *et al.* 1994). Recent research by Ryan *et al.* (1994) shows that Ca primarily reduces the negative effects of Al on root elongation, not on Ca uptake. Despite this, the Ca concentrations in the above ground parts of *C. dissectum* and *A. montana* are significantly raised by the addition of 1000 or 5000 µmol Ca l^{-1} to the nutrient solution in this experiment. A correlation between Ca in the soil solution and plant uptake was also found in the field for *C. dissectum* by Hayati and Proctor (1990). This increase in Ca concentrations in *A. montana* and *C. dissectum* correlated partly with the increase in biomass production and improved vitality of the plants. Still, the detrimental effects of Al cannot be fully reversed by high Ca levels (Figure 1), indicating that high concentrations of Al are toxic themselves to these two species.

Another cause for the reduced growth of *C. dissectum* on 5000 µmol Ca l^{-1} and 500 µmol Al $^{-1}$ might be the very high Ca concentrations in the nutrient solution, which reduce Mg concentrations in the shoots. Schimansky (1991) reported a similar negative effect of high Ca concentrations in the nutrient solutions, which added to the effects of Al toxicity.

ECOLOGICAL IMPLICATIONS

Plants in acidic habitats have to deal with extreme soil properties: high hydrogen and aluminium concentrations, low calcium concentrations, high manganese and ferrous concentrations and high ammonium to nitrate ratios (Runge & Rode 1991). The results of this experiment reveal the sensitivity of *A. montana* and *C. dissectum* to Al concentrations > 100 µmol l^{-1}. This could be one of the major causes for the recent decline of these species. The observed sensitivity is in good agreement with the results of pot experiments of Heijne (1995) and with those of a field survey by Fennema (1992). The latter showed that sites from which *A. montana* had recently became extinct, had higher exchangeable Al concentrations than sites where it was still present.

Since all the tested plants developed well in our experiments at pH=4.0, when they were grown on nutrient solution containing 50 µmol l^{-1} Al or less, we assume that toxicity of hydrogen ions is also not important for *A. montana, C. dissectum* or *C. vulgaris*.

Our results indicate furthermore that low Ca concentrations in soil might be a problem for *A. montana* and *C. dissectum*. Both species have distinctly higher Ca concentrations in the shoot than *C. vulgaris* (Table 1, Hayati & Proctor 1990, Pegtel 1994). Again, these findings confirm the limited adaptation of *A. montana* and *C. dissectum* to acidifying conditions, where *C. vulgaris* is more tolerant to the acidic conditions.

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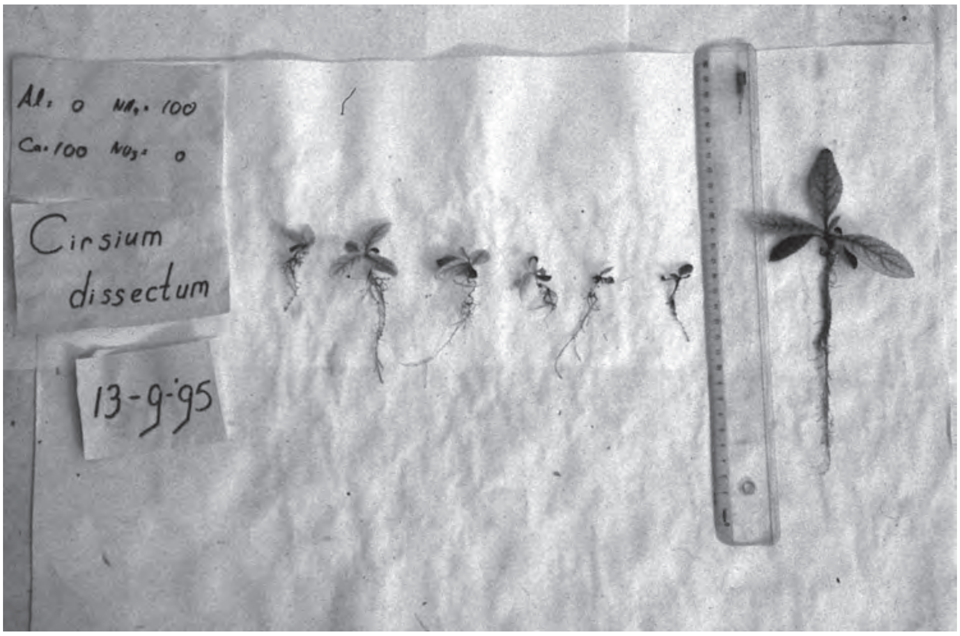
CHAPTER 5

EFFECTS OF NITROGEN NUTRITION AND ALUMINIUM ON SEEDLING DEVELOPMENT OF *ARNICA MONTANA* L. AND *CIRSIUM DISSECTUM* (L.) HILL



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ABSTRACT

Soil acidification below pH=4.5 leads to an increase in Al to phytotoxic concentrations and generally decreases nitrification rates. This causes a shift in the dominant mineral nitrogen form from NO_3^- to NH_4^+. The effects of both increasing Al concentrations and changes in mineral N nutrition have been the subject of many studies; however, information on the interactions between both is scarce. Therefore, we set up a factorial experiment in order to study the interactions between Al concentration and mineral nitrogen source (NO_3^-, NH_4^+ or a mixture). The experiments were carried out using *Arnica montana* and *Cirsium dissectum*. Both species are characteristic of acidic habitats (pH 4.5-6.0) and have been declining in the Netherlands in recent decades as a result of soil acidification. In previous studies, it was demonstrated that both were sensitive to high aluminium concentrations and to unilateral ammonium nutrition when grown without AM fungi.

Plants were grown for 5 – 7 weeks on nutrient solutions differing in Al concentration (0, 50 and 200 µM) and nitrogen form (NO_3^-, $NH_4^+ + NO_3^-$ or NH_4^+; 100 µM). Survival, growth and morphology were studied. Special attention was paid to the chemical composition of the roots and shoots, as both Al and N-nutrition are known to affect the plants' chemical composition, and nutritional imbalances may arise from high Al- or imbalanced N-nutrition.

Survival of neither species was significantly affected by N-source, Al-concentration or interactions between both. Both species showed a strong response to N-source: in general, ammonium nutrition led to a disturbed root morphology and to reduced uptake of K, Ca and Mg. *Cirsium*, in particular, was very sensitive to ammonium nutrition: in a small additional experiment it was shown that the ammonium toxicity was probably associated with an internal acidification of the plant. *Arnica* was less sensitive to NH_4^+-nutrition: plants grew best on a mixture of nitrate and ammonium, despite reduced cation uptake and stunted root formation.

No severe growth inhibition by aluminium was observed in *Arnica* or *Cirsium*. Moreover, low aluminium concentrations (50 µM) stimulated growth, which correlated with an increase in content of most nutrients. On 200 µM Al, nutrient uptake was generally inhibited, especially when NH_4^+ was the sole N-source.

Interactions between N-source and Al-concentration were rare in *Arnica*, but were frequently observed in *Cirsium*. Responses in total and specific root length (SRL) and shoot/root ratios were altered by N-Al-interactions, as was the uptake of nutrients.

The response in SRL was striking: SRL was greatly reduced when both NH_4^+ and Al were present in the nutrient solution, indicating a reduced uptake capacity under these circumstances.

The physiological and ecological implications of the results are discussed. It is concluded that soil acidification is a serious threat to both species, despite the fact that both species respond differently to changes in N-nutrition and Al.

INTRODUCTION

Acid soils ($pH \leq 4.5$) exhibit a number of specific conditions: high concentrations of hydrogen, aluminium, iron and manganese, a low concentration of base cations (especially of calcium), and often a high ammonium to nitrate ratio (Runge & Rode 1991). Only a limited number of plant species are adapted to these acid conditions. As characteristic plant species of moderately acidic soils (pH 4.5 – 6.0) are not adapted to these acid soil conditions, acidification leads to the decline of these species. This has been observed in forests, heathlands and grasslands (Van Dam *et al.* 1986, Fennema 1992, Falkengren-Grerup & Tyler 1993). In recent decades, soil acidification has occurred on a large scale as a result of atmospheric deposition of NO_x, NH_y and SO_z (Tickle *et al.* 1995, Bobbink 1998). Moreover, due to atmospheric N-deposition, nitrogen may no longer be a limiting factor in ecosystems and so may eventually lead to rather drastic changes in ecosystem functioning (Bobbink *et al.* 1998). For instance, dwarf shrub dominated heathlands may change into grasslands due to increased N-availability (Aerts & Heil 1993). It is likely that processes associated with such an increased availability of nitrogen will interfere with acidification processes, as present NO_x and NH_y levels form a substantial part of total acidifying atmospheric deposition in many West european countries (Asman *et al.* 1998, Fowler *et al.* 1998). However, as we have reported in detail on the effects of increased ammonium availibity in a former article (De Graaf *et al.* 1998), we shall limit this study to the effects of acidification at low nitrogen concentrations.

Under such acid circumstances, an increase in phytotoxic Al^{3+} and a shift in the dominant nitrogen source from nitrate to ammonium, due to inhibited nitrification in acid soils, are considered to be of major importance in the decline of many plant species (Nilgård 1985, Boxman *et al.* 1991, Runge & Rode 1991, Falkengren-Grerup & Lakkenborg-Kristensen 1994, De Graaf *et al.* 1997 1998).

Ammonium is usually the preferred mineral nitrogen source ofspecies of acid soils ($pH < 4.5$), whereas species in less acid habitats generally prefer nitrate or a mixture of ammonium and nitrate (Gigon & Rorison 1972, Falkengren-Grerup & Lakkenborg-Kristensen 1994, De Graaf *et al.* 1998). Hence, alterations in the mineral nitrogen source may have great physiological implications for plants, not least because of the fact that nitrate uptake is suppressed by external ammonium (Marschner *et al.* 1991). Moreover, ammonium which has been taken up, has to be assimilated immediately, whereas nitrate can be stored in the vacuoles. Due to the immediate assimililation, ammonium uptake is associated with a immediate demand for carbon skeletons. This may eventually lead to reduced root and shoot growth, due to a lack of carbon for other plant functions (Salsac *et al.* 1987).

Uptake of nitrate is accompanied by increased cation uptake and reduced anion uptake (Salsac *et al.* 1987). In contrast, an increase in anion uptake is often observed with ammonium uptake, but ammonium uptake competes with the uptake of other cations (Raven & Smith 1976). The reduced uptake of cations such as calcium, magnesium and potassium may lead to nutritional deficiencies (Salsac *et al.* 1987, Boxman *et al.* 1991, Pearson & Stewart 1993). Furthermore, in order to maintain charge balance, ammonium uptake is often accompanied with the excretion of protons, which may further acidify the rhizosphere (Raven & Smith 1976).

It is generally assumed that Al^{3+} is the most toxic aluminium form to plants (Foy *et al.* 1988, Ryan *et al.* 1994, Kinraide 1997). Although the mechanism of aluminium toxicity is not yet fully understood, it is clear that the roots are the primary target of aluminium (Kochian 1995, Delhaize & Ryan 1995). Membrane functions are altered, root elongation is inhibited and thereby the capacity for nutrient uptake is reduced (Rengel 1992). This may lead to nutritional disorders, especially deficiencies of calcium, magnesium and phosphorus (Foy *et al.* 1988, Boxman *et al.* 1991) and sometimes also of potassium (Foy *et al.* 1988).

Most studies on aluminium toxicity have been performed with nitrate as a sole nitrogen source, although it is known that the nitrogen source may have great impact on the development of aluminium toxicity. Many species were found to show a stronger, more negative response to aluminium with nitrate as N source than with ammonium (Cumming & Weinstein 1990, McCain & Davies 1983). Moreover, soybean appeared to be less sensitive to Al in the presence of ammonium (Klotz & Horst 1988). Contrastingly, some studies on wheat showed aluminium toxicity increased in the presence of ammonium (Taylor & Foy 1985, Taylor 1988).

One may question the ecological significance of experiments in which nitrate is the sole N source, as phytotoxic Al^{3+} is merely found in soils were the $pH<4.5$, i.e. in soils where ammonium is the dominant mineral nitrogen source. Rorison (1985) showed that the interaction between aluminium and mineral nitrogen source in different species may depend on the acidity of their natural habitat. He showed that in *Deschampsia flexuosa*, a species characteristic of acid soils, aluminium was only toxic at high concentrations with nitrate as nitrogen source. In contrast, it was observed that in *Bromus erectus*, a species which is usually found on circumneutral soils, aluminium reduced growth regardless of N source. Besides aluminium toxicity, ammonium toxicity had an even greater impact on this species. Most interesting was the growth response of *Holcus lanatus*, a species of moderately acidic to alkaline soils. Growth of this species was inhibited by aluminium with nitrate as N source, whereas growth was stimulated by aluminium when ammonium was applied.

This study will focus on interactions between mineral nitrogen source and aluminium in *Arnica montana* and *Cirsium dissectum*, both characteristic of moderately acidic soils. They occur in heathlands and grasslands (soil pH 4.5 - 6.0): *Arnica* is merely found in dry ecosystems, whereas *Cirsium* occurs in moist sites. Their recent decline is thought to be caused by soil-mediated effects of acidification (Fennema 1992, Roelofs *et al*. 1996). In previous studies (De Graaf *et al.*,1997 1998), seedlings of both species responded negatively to an increase in aluminium concentrations (with nitrate as N source) or in ammonium concentrations.

A factorial design was chosen to study the effects of different aluminium concentrations on the growth, development and chemical composition of *Arnica* and *Cirsium*, cultured with nitrate, ammonium or a mixture as N source. We always used low N-concentrations (100 mmM) in order to avoid toxicity of ammonium. With this approach, we intend to answer the following questions:

- Does the sensitivity to Al^{3+} change when plants are grown with different nitrogen sources?
- Does the sensitivity to ammonium change in the presence of aluminium?
- Is there a similarity in response of the species to aluminium and nitrogen source, as would be expected considering the acidity of the natural habitat?

MATERIAL AND METHODS

Growth and development of Arnica montana *and* Cirsium dissectum *on nutrient solutions*

Seeds of *Arnica* and *Cirsium,* which originated from populations in Dutch nature reserves, were germinated at room temperature on two layers of wetted filter paper. The seedlings were transferred when root length was approximately 2 cm, at this stage, *Arnica* seedlings had two fully unfolded cotedylons, whereas in *Cirsium* seedlings the first two leaves were visible. In order to acclimate seedlings, they were placed on a complete nutrient solution containing 50 µM NO_3^- and 50 µM NH_4^+, but no aluminium (see below and Table 1, solution 4). This acclimation period lasted 9 and 14 days for *Arnica* and *Cirsium* respectively. Seedlings which died during acclimation were replaced, as mortality was considered an artefact of transfer.

The plants were grown in a water culture system, made up of 2 l opaque containers (referred to as ‘plant containers’), in which the nutrient solution was continously refreshed (1.2 l per 24 h). Each plant container was connected to a separate storage container, filled with nutrient solution. A continuous air flow through the solution prevented the development of anoxic conditions. Four plant containers per treatment per species were used, each with cultures of either 9 *Arnica* or 6 *Cirsium* individuals. The difference in plant numbers compensated for differences in growth rate, which is higher in *Cirsium* than in *Arnica*.

Table 1 NO_3^-, NH_4^+, Al^{3+}, Na^+ and Cl^- concentrations in the nutrient solutions in µmol l^{-1}.

Treatment	**NO_3^-**	**NH_4^+**	**Al_3^+**
1	100	0	0
2	100	0	50
3	100	0	200
4	50	50	0
5	50	50	50
6	50	50	200
7	0	100	0
8	0	100	50
9	0	100	200

The whole system was placed in a completely randomised block design in a climate chamber, in which temperature fluctuated during the photo-period between 24°C and 28°C (16 h, light intensity 100 ± 10 µE m^{-2} s^{-1}, relative humidity 40-50 %) and between 16 °C and 21 °C during darkness (8 h, relative humidity 85-90%).

The nutrient concentrations in the solutions (Table 1) were low, in order to represent natural conditions (De Graaf *et al.* 1994), with the exception of phosphate, as this nutrient may be limiting under natural conditions. Macronutrients: 100 µM Ca^{2+}, 100 µM Mg^{2+}, 200 µM K^+ (except for *Arnica*, where 150 and 100 µM K^+ were applied in the mixed N treatments and in the NH_4^+ only treatments), 100 µM SO_4^{2-}, 200 µM PO_4^{3-}; the concentrations of the other macronutrients are given in Table 1. As aluminium was applied as $AlCl_3$ and NaCl was used to correct for ionic balance, Na^+ and Cl^- concentrations were variable (Na^+: 67-617 µM, Cl^-: 567-1067 µM). Micronutrients: 0.27 µM Fe^{2+} (applied as Fe-EDTA), 0.0007 µM Zn^{2+}, 0.0008 µM Mn^{2+}, 0.0002µM Cu^{2+}, 0.0008 µM Mo, 0.0008 µM H_3BO_3 and 0.0003 µM thiaminedichloride. The nutrient solutions in the storage containers were sampled weekly and checked for differences in nutrient concentrations, but none were found. The pH of the solutions in the storage containers was adjusted twice a week to 4.0 by the addition of HCl or NaOH. pH fluctuations in the plant containers were not controlled as they were considered a treatment effect, caused by plant nutrient uptake.

During the experiment, survival of the individuals was monitored weekly, as was the pH in the plant containers. *Arnica* and *Cirsium* were harvested 61 resp. 34 days after the treatments had began. At this time, some containers were fully covered by leaves and competition for light became apparent. Roots and shoots were separated and both were visually inspected for abnormal morphology and colour. Roots were thoroughly rinsed in demineralised water, and total root length per plant container was then measured, using a Comair© root length scanner. Due to equipment failure, root length of *Arnica* could only be measured once in most treatments; in the treatments with solely NH_4^+ as N source and 50 or 200 µM Al (treatments 8 and 9), root length could not be determined at all.

Dry weights of roots and shoots of individual plants were determined after a drying period of 24 h at 70°C.

Chemical analyses

For analyses of the chemical composition of shoots and roots, dried plant material of each species per treatment was pooled per container (n=4), ground in liquid nitrogen and dried again (70° C, 24 h). 50 mg of this dry material was digested in 5 ml 95-97% H_2SO_4 and 2 ml 30% H_2O_2 (Van Dijk & Roelofs 1988). P, Ca and Mg concentrations were measured using an ICP (type Spectroflame, simultaneous emmision spectrometer) and K concentrations were quantified with flame photometry (Technicon Flame photometer IV). 20 mg of dry, ground plant material was used for the determination of N concentrations, using a CNS analyzer (type NA 1500, Carlo Erba Instruments).

Internal pH of Cirsium dissectum

Seedlings of *Cirsium* were cultured on a 10% strength Hoagland solution in a water culture system, under similar conditions as the growth and development experiment. After 17 weeks, the Hoagland nutrient solution was replaced by low nutrient solutions, containing either NO_3^- or NH_4^+ as nitrogen source (comparable to the treatments without aluminium in the former experiment, with SO_4^{2-} being 500 µM). After 14 days of growth on these solutions, roots and shoots were separated and 0.3-0.6 g fresh material was ground in liquid nitrogen. 0.3 to 0.6 ml of demineralised water was added; after thawing, the pH of the homogenate was immediately measured using a glass-pH-electrode with a 1.7 mm tip diameter (after Lang & Kaiser 1994). Five replicates were used.

Statistical and graphical analyses

Survival of plants, growth of surviving plants (total plant dry weight, dry weight of roots and shoots separately, root length and specific root length) and chemical composition of shoots and roots (concentration and content) were statistically analysed by a two-way analysis of variance (GLM procedure, SAS 6.0), with nitrogen source and aluminium concentration as class variables.

A graphical method after Timmer and Stone (1978) was used to depict the effects of nitrogen source and aluminium concentration on the performance of the plant species. Scatterplots of element concentrations (µmol g^{-1}) in plants versus plant nutrient contents (µmol; concentration x dry weight) were made; in each plot five categories could be distinguished, according to the response of the plant relative to the control. When both concentration and content of an element increased in proportion to the control, the element was considered to be less limiting. In contrast, when both concentration and content decreased in proportion to the control, increased limitation was likely. When the increase in concentration was greater than that of the content, this was seen as luxury consumption. Dilution effects were characterised by an increase in content but a decrease in concentration. A reduction in content and an increase in concentration may either be due to toxicity of the plotted nutrient or to limitation of another nutrient.

RESULTS

Morphology, survival and biomass

The morphology of *Arnica* and *Cirsium* was largely affected by nitrogen source and to a lesser extent by aluminium: we observed large differences in root morphology as well as in the colour of the leaves. Both species developed large, fully branched root systems when nitrate was applied as the sole nitrogen source. In contrast, when ammonium was applied (with or without nitrate), the development of the lateral roots was inhibited, leading to the formation of stunted roots. In both species, the number of stunted roots increased with an increase in the proportion of ammonium in the nutrient solution.

As a result of increasing aluminium concentrations, some plants developed less healthy root systems, showing similar morphological responses to aluminium as to ammonium. However, the effects of aluminium on root morphology were less pronounced than those of ammonium, and were only observed with ammonium as a sole N source in *Cirsium* and in the presence of nitrate (with or without ammonium) in *Arnica*.

Arnica and *Cirsium* developed yellow leaves when ammonium was supplied as N source. In *Cirsium,* almost all leaves were yellow whenever ammonium was present in the nutrient solution. In *Arnica*, more yellow leaves were observed as the proportion of ammonium in the nutrient solution increased. In the sole ammonium treatments, approximately 95 % of the leaves were yellow, regardless of aluminium concentrations. No additional negative effects of aluminium to the ammonium effects on the discolouration of the leaves were noted. In contrast, in the 50 µM Al treatment with nitrate as N source, both species developed more healthy, green leaves than in any other treatment.

Despite the large effects of nitrogen on roots and shoots, survival of either species was not significantly affected by nitrogen, aluminium or an interaction of both. Nearly all *Cirsium* plants survived, but in *Arnica*, a tendency was observed towards higher mortality on ammonium than on nitrate (Table 2). A mixed N supply led to intermediate survival. Moreover, highest survival of *Arnica* was noted in treatments

Table 2 Mean survival percentage after 63 days of *Arnica* on nutrient solutions differing in nitrogen source and in aluminium concentration. No significant effects of aluminium concentration nor of nitrogen source were detected ($p<0.05$).

	Al=0	Al=50	Al=200
NO_3^-	75	75	66.7
$NO_3^- + NH_4^+$	72.2	63.9	72.2
NH_4^+	61.1	63.9	55.6

without aluminium, with nitrate being the sole N source (75.0%). The lowest survival was recorded in the 200 µM Al treatment with ammonium as a N source (55.6%).

Nitrogen source also affected root and shoot biomass of both species (Table 3, 4). In contrast to what would be expected from the large reduction in healthy roots and the yellowing of the leaves in the presence of ammonium, *Arnica* plants had a higher biomass on NH_4^+-containing solutions (Table 3). Shoot biomass of plants grown on ammonium nutrition (mixed or sole treatments) was more than double the shoot biomass of nitrate grown plants. The largest plants developing on mixed N source. The increase in root biomass on ammonium containing solutions was less than that of shoot biomass (130 – 150 %), resulting in an increased shoot/root ratio.

In contrast to *Arnica*, *Cirsium* showed a significant decrease in both root and shoot biomass whenever ammonium was present in the nutrient solution (Table 3). We did not observe significant effects of nitrogen source on shoot/root ratios in this species.

Aluminium stimulated biomass formation of roots and shoots in *Cirsium*, with an optimum at 50 µM Al (Table 3, 4). Biomass of *Arnica* was not significantly affected by the addition of aluminium, although a small increase of root and shoot dry weight was observed in the 50 µM Al treatments with nitrate present in the nutrient solution. In contrast, both root and shoot dry weights of *Arnica* decreased with increasing aluminium concentration when ammonium was the sole N source, although this interaction between N source and aluminium concentration was not statistically significant (Table 4). Neither such interaction was found to affect root and shoot biomass of *Cirsium*, although shoot dry weights increased less in reponse to 50 µM Al with increasing ammonium proportion in the nutrient solution. Shoot/root ratios of neither *Arnica* nor *Cirsium* showed a significant response to aluminium.

There was however a significant N*Al interactive response of total and specific root length in *Cirsium* (SRL: total root length divided by root dry weight; Table 3, 4). While 50 µM Al increased total root length up to 300 % in the presence of nitrate as the sole N source, total root length increased only to 150 % when NH_4^+ was added to the nutrient solutions containing 50 µM Al (Table 3). The interaction between nitrogen source and aluminium was even more pronounced in the response of SRL (Table 3). Without aluminium, the SRL of *Cirsium* was independent from nitrogen source.

Table 3 Plant dry weight (root, shoot and total), shoot/root ratio (based on dry weight), root length and specific root length. Root length and specific root length are the sum of root length per plant compartment. The response to aluminium per N source is indicated as the percentage of each factor and is given between brackets. *: missing value. Mean of 4 replicates (#: N=3). Root length and SRL in *Arnica montana* were only measured in one replicate.

N source	Aluminium (µM)	Dw_{root} (mg)	Dw_{shoot} (mg)	Dw_{total} (mg)	Shoot/root ratio	Root length (m)	SRL (m/mg)
Arnica montana							
NO_3^-	**0**	27.2	88.8	116.1	3.62	13.4	0.091
NO_3^-	**50**	31.4	120.1	151.4	6.16	19.3	0.173
NO_3^-	**200**	27.8	95.4	123.2	3.66	8.0	0.111
$NO_3^- + NH_4^+$	**0**	41.4	207.7	249.1	6.37	12.5	0.074
$NO_3^- + NH_4^+$	**50**	52.7	243.5	294.9	8.84	20.9	0.197
$NO_3^- + NH_4^+$	**200**	35.3	204.1	239.3	7.15	15.7	0.101
NH_4^+	**0**	36.1	202.1	238.2	6.00	9.8	0.069
NH_4^+	**50**	26.9	204.1	230.9	8.07	*	*
NH_4^+	**200**	27.5	176.2	203.6	15.41	*	*
Cirsium dissectum							
NO_3^-	**0**	23.9	64.2	92.4	3.11	15.9	0.647
NO_3^-	**50**	$61.3^{\#}$	$211.7^{\#}$	$273.0^{\#}$	$4.08^{\#}$	$48.0^{\#}$	$0.822^{\#}$
NO_3^-	**200**	31.3	77.2	108.5	2.65	13.8	0.407
$NO_3^- + NH_4^+$	**0**	7.5	23.9	31.3	4.02	4.5	0.622
$NO_3^- + NH_4^+$	**50**	$27.8^{\#}$	$48.5^{\#}$	$76.3^{\#}$	$2.22^{\#}$	$6.6^{\#}$	$0.250^{\#}$
$NO_3^- + NH_4^+$	**200**	15.4	31.6	47.0	3.68	3.0	0.197
NH_4^+	**0**	5.2	20.1	25.3	3.89	3.2	0.634
NH_4^+	**50**	15.0	30.6	45.5	2.62	4.2	0.267
NH_4^+	**200**	14.5	30.3	44.8	2.40	3.4	0.238

However, in the presence of aluminium, SRL was severely reduced whenever ammonium was supplied as a N source, whereas on nitrate nutrition, low aluminium concentrations increased SRL. 200 µM aluminium led to a reduction of 37% in SRL. Similar interactions between N source and aluminium concentration were seen in the shoot/root ratio of *Cirsium*.

Table 4 Anova table of the effects of N source, aluminium concentration and the interactive effect on mean plant dry weight (root, shoot and total), shoot/root-ratio (based on dry weights) root length and specific root length. Root length and SRL of *Arnica montana* are not tested as N=1. Significance value P<0.001:***; P<0.01:**; P<0.05:*; ns = not significant at P<0.05. The significance value is given when 0.05<P<0.10.

	Dw_{root}	Dw_{shoot}	Dw_{total}	shoot/root	Root length	SRL
Arnica montana						
N	0.10	***	***	*	.	.
Al	ns	ns	ns	ns	.	.
N*Al	ns	ns	ns	ns	.	.
Cirsium dissectum						
N	**	***	***	ns	***	***
Al	*	*	*	ns	*	***
N*Al	ns	0.08	ns	*	*	***

SRL of *Arnica* was reduced with an increasing ammonium proportion in the solution (0 µM Al; Table 3). Addition of Al stimulated SRL in both the nitrate and the mixed N-treatments. The total root length development was only stimulated by 50 µM Al with nitrate as the sole N source; root length was reduced to 60% on 200 µM Al. Such a reduction in total root length by Al was not found in the mixed N-treatment.

Due to equipment failure, the effects of aluminium on plants grown with ammonium as the sole N source could not be studied.

Chemical composition of roots and shoots

Both the concentration and the content (concentration x dry weight) of most macronutrients were affected by nitrogen form and aluminium concentration of the nutrient solution. However, interactions between both occurred to a lesser extent (Table 5, 6). In this paragraph, we shall firstly describe the separate effects of nitrogen nutrition and external aluminium concentration on the nutrient concentration in roots and shoots. Thereafter, the effects on total plant uptake, as reflected in the nutrient contents, will be described. Finally we will describe interactions between nitrogen nutrition and aluminium concentration, firstly on plant nutrient concentrations followed by the effects on the plant contents of the macronutrients.

Effects of N source

Without aluminium being added to the nutrient solution, the nitrogen concentration of roots and shoots of both *Arnica* and *Cirsium* increased with an increasing

Table 5 Chemical composition of roots and shoots of *Arnica montana* and *Cirsium dissectum*, as affected by N source and aluminium concentration. Mean values and SE, N=4. Missing value: .

N source	**Aluminium**	**Concentration (% of dry weight)**						**Content (mg)**					
	(µM)	**N**	**Al**	**Ca**	**Mg**	**K**	**P**	**N**	**Al**	**Ca**	**Mg**	**K**	**P**
Arnica montana, root													
NO_3^-	**0**	1.99	0.02	0.31	0.29	3.88	1.92	0.55	0.00	0.09	0.08	1.89	0.54
NO_3^-	**50**	2.18	0.46	0.28	0.32	4.80	3.09	0.71	0.14	0.09	0.12	1.71	1.10
NO_3^-	**200**	2.03	0.52	0.20	0.21	4.13	2.48	0.55	0.14	0.05	0.06	1.07	0.65
$NO_3^- + NH_4^+$	**0**	3.29	0.01	0.21	0.16	1.76	1.07	1.26	0.00	0.08	0.07	0.70	0.39
$NO_3^- + NH_4^+$	**50**	3.13	0.16	0.16	0.17	2.60	2.13	1.52	0.08	0.08	0.09	1.36	1.05
$NO_3^- + NH_4^+$	**200**	2.88	0.28	0.16	0.15	2.71	2.01	0.99	0.10	0.06	0.05	0.95	0.71
NH_4^+	**0**	4.68	0.02	0.20	0.12	1.34	0.95	1.69	0.01	0.07	0.04	0.48	0.34
NH_4^+	**50**	5.70	0.16	0.16	0.14	1.56	1.64	1.53	0.04	0.04	0.04	0.42	0.44
NH_4^+	**200**	5.41	0.30	0.16	0.11	1.15	1.19	1.75	0.08	0.04	0.03	0.36	0.37
Arnica montana, shoot													
NO_3^-	**0**	2.47	0.00	1.23	0.54	5.56	1.54	2.19	0.00	1.11	0.49	5.01	1.39
NO_3^-	**50**	2.63	0.01	0.95	0.44	6.56	1.71	3.19	0.01	1.22	0.54	8.26	2.11
NO_3^-	**200**	2.74	0.04	0.64	0.32	6.83	1.46	2.74	0.04	0.63	0.32	6.67	1.39
$NO_3^- + NH_4^+$	**0**	2.59	0.00	0.59	0.36	3.78	0.75	5.02	0.00	1.18	0.71	7.37	1.63
$NO_3^- + NH_4^+$	**50**	3.04	0.01	0.36	0.29	.	1.39	7.26	0.04	0.86	0.69	.	3.30
$NO_3^- + NH_4^+$	**200**	3.07	0.05	0.39	0.25	5.93	1.34	6.32	0.09	0.77	0.51	11.17	2.74
NH_4^+	**0**	3.78	0.00	0.52	0.35	3.37	0.83	7.6	0.00	1.04	0.69	6.81	1.66
NH_4^+	**50**	4.34	0.02	0.42	0.35	5.38	1.37	8.74	0.04	0.84	0.69	10.88	2.74
NH_4^+	**200**	4.46	0.03	0.35	0.30	4.93	1.11	7.58	0.06	0.57	0.49	8.51	1.92
Cirsium dissectum, root													
NO_3^-	**0**	2.75	0.02	0.33	0.12	3.05	1.28	0.81	0.00	0.06	0.02	0.53	0.23
NO_3^-	**50**	2.57	0.29	0.26	0.17	5.52	2.08	1.45	0.14	0.16	0.11	3.34	1.31
NO_3^-	**200**	2.64	0.57	0.20	0.09	3.74	1.56	0.83	0.17	0.06	0.03	1.24	0.50
$NO_3^- + NH_4^+$	**0**	3.04	0.04	0.30	0.09	2.47	1.40	0.35	0.00	0.02	0.01	0.15	0.09
$NO_3^- + NH_4^+$	**50**	3.19	0.26	0.19	0.07	2.27	1.16	0.95	0.07	0.06	0.02	0.71	0.36
$NO_3^- + NH_4^+$	**200**	3.14	0.40	0.16	0.06	2.28	1.18	0.48	0.06	0.02	0.01	0.35	0.18

continued at page 90

Table 5 Continued

N source	Aluminium	Concentration (% of dry weight)						Content (mg)					
	(µM)	N	Al	Ca	Mg	K	P	N	Al	Ca	Mg	K	P
NH_4^+	0	4.66	0.03	0.51	0.10	2.59	0.80	0.35	0.00	0.02	0.01	0.13	0.06
NH_4^+	50	3.62	0.28	0.16	0.05	2.28	1.29	0.60	0.04	0.02	0.01	0.35	0.20
NH_4^+	200	3.88	0.44	0.12	0.05	2.09	1.51	0.56	0.06	0.02	0.01	0.31	0.22
Cirsium dissectum, shoot													
NO_3^-	0	3.58	0.00	2.35	1.03	3.63	0.80	2.27	0.00	1.54	0.66	2.51	0.49
NO_3^-	50	3.26	0.02	2.04	0.96	5.42	0.54	6.15	0.02	4.24	2.00	11.57	1.00
NO_3^-	200	3.68	0.03	1.17	0.57	6.18	0.55	2.78	0.03	0.92	0.45	4.86	0.44
$NO_3^- + NH_4^+$	0	4.59	0.00	1.86	0.94	4.32	1.16	0.96	0.00	0.39	0.19	0.93	0.25
$NO_3^- + NH_4^+$	50	4.35	0.02	1.24	0.64	5.23	0.78	2.17	0.01	0.61	0.31	2.63	0.39
$NO_3^- + NH_4^+$	200	4.12	0.04	1.01	0.53	5.35	0.73	1.32	0.01	0.31	0.16	1.71	0.23
NH_4^+	0	5.24	0.00	1.71	0.85	3.91	1.09	1.19	0.00	0.38	0.119	0.82	0.24
NH_4^+	50	4.28	0.02	1.03	0.57	4.67	0.85	1.32	0.01	0.32	0.18	1.47	0.26
NH_4^+	200	4.42	0.04	1.01	0.53	4.90	0.82	1.33	0.01	0.31	0.16	1.52	0.25

proportion of ammonium in the nutrient solution (Table 5). In contrast, cation concentrations, including aluminium, decreased in both roots and shoots when ammonium was applied as nitrogen source. We observed two exceptions: in *Cirsium* roots, the calcium concentration was not significantly affected by nitrogen source, and neither was the potassium concentration in the shoots. In general, cation concentrations differed especially between the sole nitrate and both ammonium-containing treatments. A further reduction in cation concentrations between the mixed N and the sole ammonium treatment was only observed in some cases (e.g. potassium in *Arnica* roots and *Cirsium* shoots).

Nitrogen nutrition also affected the phosphorus concentration in the plants; in *Arnica*, P concentrations were particularly higher in the nitrate grown plants (in both roots and shoots), whereas in *Cirsium*, phosphorus concentrations reached highest values in the mixed N treatment.

Both the nitrogen content and the nitrogen concentration in *Arnica* increased with addition of ammonium to the nutrient solution. In contrast, the nitrogen content of *Cirsium* decreased when ammonium was present. In *Cirsium*, the contents of calcium, magnesium and potassium also decreased in the presence of ammonium, as was the potassium content in *Arnica*. In the latter plant species, the calcium content was not significantly affected by nitrogen source, but calcium contents were generally reduced in the presence of ammonium. Shoot magnesium content, however, increased in the ammonium treatments, as opposed to root magnesium contents.

The phosphorus contents of the *Arnica* roots showed a similar response to the nitrogen source as did the P concentrations: they decreased on ammonium nutrition. In the shoots however, the opposite was observed: here the P content was higher on ammonium nutrition. In both the roots and shoots of *Cirsium*, the phosphorus content of plants grown with ammonium was lower than the P content of plants grown with nitrate. The P content of the plants cultured with both ammonium and nitrate hardly differed of that of ammonium grown plants.

EFFECTS OF EXTERNAL ALUMINIUM CONCENTRATIONS

In general, the aluminium concentration in the nutrient solution affected the chemical composition of shoots more than roots (Tables 5 and 6). Plant aluminium

Table 6 Anova table of the effects of N source, aluminium concentration and the interactive effect on the chemical composition of roots and shoots of *Arnica montana* and *Cirsium dissectum*. Mean concentrations (expressed as % of dry weight) and contents are given in Table 6. Significance value P<0.001:***; P<0.01:**; P<0.05:*; ns = not significant at P<0.05. The significance value is given when 0.05<P<0.10.

	Concentration (% DW)						**Content (mg)**					
	N	**Al**	**Ca**	**Mg**	**K**	**P**	**N**	**Al**	**Ca**	**Mg**	**K**	**P**
Arnica montana												
root												
N	***	***	***	***	***	***	***	*	ns	0.06	**	*
Al	ns	***	**	*	0.06	***	ns	***	0.07	ns	0.20	*
N*Al	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Shoot												
N	***	ns	***	***	***	***	***	0.10	ns	*	ns	*
Al	*	***	***	***	***	***	ns	***	*	*	0.06	**
N*Al	ns	ns	***	**	ns	*	ns	ns	ns	ns	ns	ns
Cirsium dissectum												
Root												
N	***	*	ns	***	***	***	0.10	***	**	**	**	*
Al	ns	***	***	***	*	**	ns	***	0.06	*	*	*
N*Al	ns	*	ns	***	***	**	ns	*	ns	0.09	0.09	ns
Shoot												
N	***	ns	***	***	ns	***	***	**	***	***	***	***
Al	*	***	***	***	***	***	**	***	*	*	*	*
N*Al	ns	ns	***	***	ns	ns	*	ns	*	*	0.08	ns

concentrations increased in both *Arnica* and *Cirsium* with increasing aluminium concentrations in the solution. Shoot nitrogen concentrations in *Arnica* were mostly increased by the aluminium treatments. In contrast, nitrogen concentrations in roots and shoots of *Cirsium* decreased with increasing aluminium concentrations in the nutrient solution (Table 6). The calcium and magnesium concentrations were also decreased by aluminium in both species. However, potassium concentrations in the plants were, in general, increased by the presence of aluminium in the nutrient solution. In shoots of both *Arnica* and *Cirsium*, the highest potassium contents were measured in the 50 µM Al-treatments. This phenomenon was also seen in the phosphorus concentrations of *Arnica*., but the phosphorus concentrations in *Cirsium* shoots decreased when aluminium was present in the nutrient solution. The response of phosphorus concentrations in the *Cirsium* roots was variable, highly depending on nitrogen source.

The calcium contents in the shoots and roots of *Arnica* decreased with an increase in external aluminium, as were the calcium concentrations. In *Cirsium*, however, the total uptake of calcium and magnesium was greatest at 50 µM Al, as was expressed by the contents of these nutrients. Especially with nitrate or mixed N-nutrition, calcium and magnesium contents more than doubled in the presence of 50 µM Al in the nutrient solution. However, at high (200 µM) aluminium concentration in the nutrient solution, plant calcium and magnesium contents were usually similar or below those of the 0 µM Al treatment. Plant potassium contents were rather variable among both species and roots and shoots, although the highest potassium contents were often measured in the 50 µM Al treatment. Phosphorus contents were always highest in the 50 µM Al treatment.

INTERACTIONS BETWEEN NITROGEN SOURCE AND EXTERNAL ALUMINIUM

Although significant effects were observed on both the concentration and content of most macronutrients in the plants by nitrogen source and aluminium concentration, statistically significant interactions between nitrogen and aluminium treatments occurred less frequently (Table 6). Moreover, the interpretation of the interactions is difficult and may vary between concentrations and contents for a certain macronutrient. For instance, interactions between nitrogen and aluminium in *Arnica* occurred in nutrient concentrations, but not in content. The use of scatter plots of nutrient content vs concentrations give a better insight in the interactions between aluminium concentrations and nitrogen source. By using these scatter plots, it is possible to distinguish between nutrient limitation, luxury consumption and dilution effects (Figure 1). Also, a situation may occur in which the concentration of an element increases in the plant, whereas the content of that element is decreased. This may either be caused by toxicity of that element or by limitation of another element.

Such graphics of shoot nitrogen show that a shift from nitrate to ammonium in *Arnica* decreased N-limitation (Figure 1). As the pattern in all nitrogen sources was

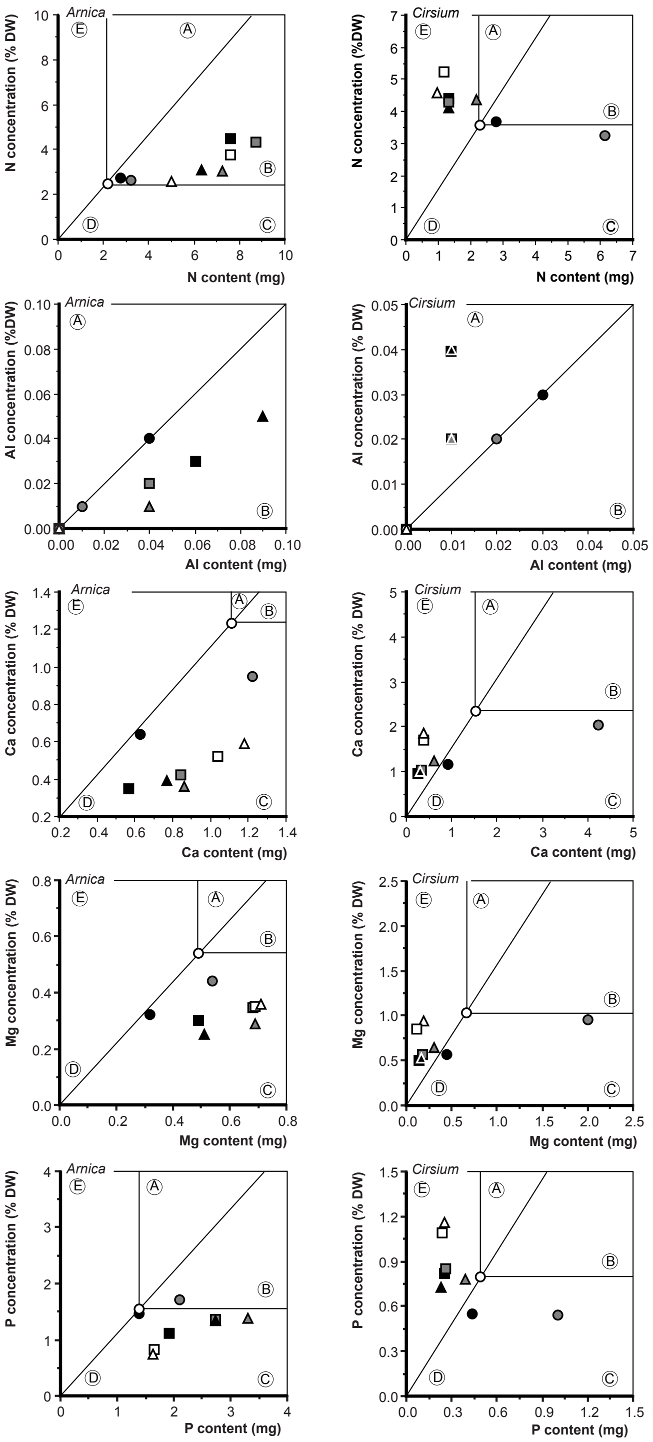

Figure 1 Scatterplots of concentration (%DW) against content of nitrogen, aluminium, calcium and magnesium response in *Arnica* and *Cirsium.* Treatments: circles: NO$_3^-$, triangles: mixed N, squares: NH$_4^+$; white symbols: Al=0, shaded symbols: Al=50 µM, dark symbols: Al=200 µM. A: luxury consumption, B: decreased limitation, C: dilution, D: increased limitation, E: toxicity or serious limitation by another element. All treatments are compared to the NO$_3^-$, Al=0 treatment.

similar, regardless of external aluminium concentration, it was clear that no N-Al-interaction exists. Contrasting to *Arnica*, a shift from nitrate to ammonium led to toxicity of ammonium in *Cirsium* (Figure 1). The plots indicated that ammonium toxicity is somewhat suppressed by 50 µM Al on mixed N sources, but with ammonium as sole nitrogen source, no suppression of the toxicity was seen with by low concentrations of aluminium.

Both in the shoots of *Arnica* and *Cirsium*, an increase in external aluminium concentration indicated decreased limitation of aluminium, not toxicity. In contrast, calcium limitation was increased in both species by a shift in nitrogen source from nitrate to ammonium and by an increase in aluminium concentration in the nutrient solution (Figure 1). It was also be observed that the increased calcium limitation in *Cirsium* was caused primarily by a shift from nitrate to ammonium nutrition rather than by an increase in aluminium. Moreover, aluminium affected both concentration and content to a greater extent with nitrate as a sole N source than when ammonium was applied with the nutrient solution. In *Arnica*, especially the calcium concentration was decreased in mixed N and ammonium treatments.

Effects of magnesium were similar to those of calcium in *Cirsium*, hence indicating that magnesium may become increasingly limiting with ammonium nutrition and high external aluminium concentrations. In *Arnica*, an increase in aluminium concentration affected shoot magnesium in a similar way as it affected shoot calcium: by an increased limitation. The effects of nitrogen source on plant magnesium were however, rather different: magnesium in plant was merely diluted when N nutrition shifted from nitrate to ammonium. Especially on 200 µM aluminium, only the shoot content was changed, whereas the shoot magnesium concentration had hardly changed.

pH-effects in nutrient solutions and in Cirsium

The pH of the nutrient solution in the plant containers of both species was affected by nitrogen source, aluminium concentration and time (N*time: $p<0.0001$, Al*time: $p<0.0001$), and by interactions between those parameters (N*Al*time: $p<0.001$; Figure 2). The pH-effects occurred despite the fact that we used a water culture system in which the nutrient solution was continously refreshed and hence, hydrogen concentrations would have been diluted throughout the experiment. The differences in pH of the nutrient solution were especially obvious during the last weeks of the experiment, when plants were large and occupied a considerable area of the plant containers.

In both the *Arnica* and *Cirsium* plant containers, the pH of the nutrient solution was higher when nitrate was applied as sole nitrogen source and plants were cultured without aluminium or on nutrient solutions containing 50 µM Al. In the presence of 200 µM Al and nitrate being the sole N source, the pH hardly differed from 4.0 throughout the experiment.

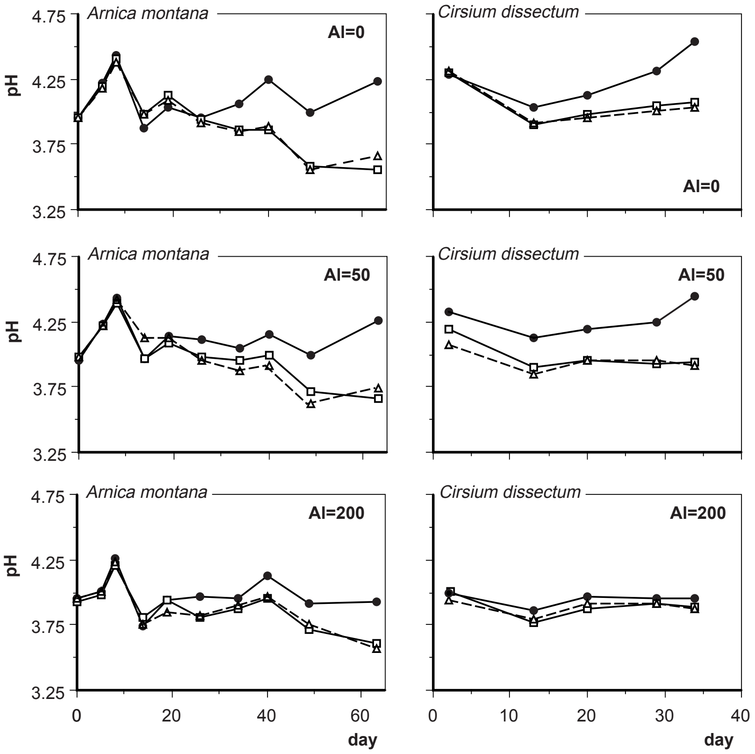

Figure 2 Time course of pH in the plant containers, planted with *Arnica* (left) or *Cirsium* (right) on different nutrient solutions. Means of four replicates. Solid line, circles: NO_3^--nutrition, solid line, squares: mixed N nutrition, dashed line, triangles: NH_4^+-nutrition.

In each aluminium treatment, the two ammonium containing treatments caused an identical pH course in the plant containers. Remarkably, however, there was a large difference in response of *Arnica* and *Cirsium* to this ammonium nutrition: *Arnica* plants cultured with ammonium, lowered the pH of the nutrient solution to values below 4.0, regardless of Al treatment. In contrast, *Cirsium* plants grown on ammonium containing nutrient solutions caused little effect to the solution pH. Only in the 200 µM Al treatment, pH values varied around 3.9, but as this was also observed in the nitrate treatment, it is likely that this was due to the aluminium in the solution, rather than to the nitrogen source.

In addition to the observation that *Cirsium* did not lower the solution pH when supplied with ammonium, as was expected, a small experiment was carried out to study the effects of nitrogen source on the plants' internal pH. Therefore, young plants were cultured for two weeks on nutrient solutions containing either ammonium or nitrate as nitrogen source. Subsequently, the roots and shoots of the plants were

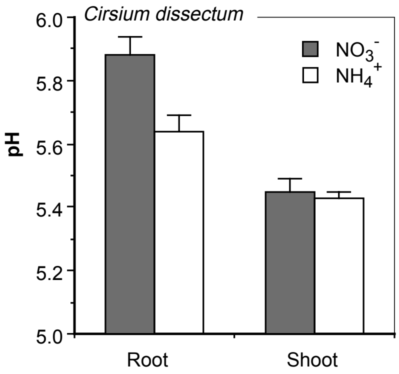

Figure 3 pH (mean ± SE) of the root and shoot homogenates of *Cirsium*, cultured on nitrate or ammonium nutrition. N=5.

ground and the pH of the homogenates was measured (see material and methods for detailed description).

In the root homogenates, the pH was significantly lower when plants were cultured with ammonium instead of nitrate (Figure 3: $p<0.0001$). The pH of shoot homogenates was not, however, affected by nitrogen source ($p<0.2694$).

DISCUSSION

Nutritional aspects of nitrogen nutrition and aluminium

Despite the extensive efforts made in studying the effects of both aluminium and nitrogen source on the nutitional status of plants, little is known about the long-term effects of the interaction between aluminium and nitrogen source on whole plants in general, and on the chemical composition of roots and shoots in particular. Most studies on aluminium-nitrogen interactions describe short-term (hours or 1-2 days) nutrient uptake experiments (Keltjens 1988, Nichol *et al.* 1993), or focus on increased root exposure to aluminium on nitrate nutrition, due to the increased influx of cations when nitrate is taken up (Cumming & Weinstein 1990, Nichol *et al.* 1993, Cumming & Brown 1994). The authors of such studies hypothesise that the increased exposure to aluminium could eventually lead to increased aluminium toxicity. Another frequently discussed topic on nitrogen-aluminium interactions is the question of whether the increased pH of the rhizosphere due to nitrate nutrition may enhance aluminium tolerance (Taylor & Foy 1985, 1988, Klotz & Horst 1988, Miyasaka *et al.* 1989).

However, it is known that nutritional disorder, originating from increased ammonium uptake following imbalanced nitrogen nutrition (Nihlgård 1985, Van Dijk & Roelofs 1988, De Graaf *et al.* 1998) or from aluminium exposure (Foy 1988, Boxman *et al.* 1991, De Graaf *et al.* 1997), can play an important role in the deterioration of ecosystems which are affected by soil acidification. Hence, we shall focus in our discussion on nutritional imbalances in order to understand the aluminium-nitrogen interactions in *Arnica* and *Cirsium* and attempt to link these to their recent decline.

This study describes the results of long-term experiments, performed on nutrient solutions which closely resemble the natural soil solution (De Graaf *et al.* 1994, Roelofs *et al.* 1996). As it is known that the composition of the nutrient solution may greatly influences processes involved in nutrient uptake and Al toxicity (e.g. McCain & Davies 1983), it is essential to use nutrient solutions which are comparable to the soil solution (Falkengren-Grerup 1994), especially when one would attempt to relate the results to field conditions. Previous experiments (De Graaf *et al.* 1997 1998) showed that by using such nutrient solutions, the chemical compositions of *Arnica* and *Cirsium* (control treatments) were generally equivalent to those of plants grown in the field, which is in our opinion a prerequisite for the comparison of solution studies with field processes. In this study, we used basically the same nutrient solutions as in the previous experiments (De Graaf *et al.* 1997 1998).

Many plant species predominantly take up ammonium from mixed, mineral nitrogen sources (Vessey *et al.* 1990, Marschner 1991, Arnold & Van Diest 1991, Troelstra *et al.* 1995). Uptake of ammonium and nitrate may have distinct effects on the pH of the nutrient solution, as ammonium uptake is usually accompanied by proton excretion, whereas nitrate uptake is coupled to OH^--excretion (Raven & Smith 1976). Thus, the pH of the solution in the plant containers can be used as an indicator of preferential nitrate or ammonium uptake (Chaillou *et al.* 1991). In this experiment, the pH of the nutrient solutions in the mixed N-treatments was equivalent to that of the NH_4-treatments but differed from the sole NO_3^--treatments, thereby indicating preferential ammonium uptake from mixed N-nutrition by *Arnica* and *Cirsium* (Figure 2). Aluminium also affected the pH of the nutrient solutions, but there are no indications that the predominant ammonium uptake was altered by aluminium (Figure 2).

Despite the fact that *Cirsium* predominantly took up ammonium in this experiment, the plants suffered from severe ammonium toxicity (Table 3). Ammonium effects were dominant over the effects of aluminium. A rather similar dominating response of ammonium nutrition on plant growth has been observed in *Bromus erectus* (Rorison 1985), a species naturally occurring in habitats with circumneutral pH.

In contrast to *Cirsium*, *Arnica* developed more biomass when supplied with ammonium than with nitrate (Table 3). The growth stimulation on NH_4^+ containing nutrient solutions, might be due to a decreased nitrogen limitation (Figure 1). This is in agreement with the suggestion of Troelstra *et al.* (1995) who stated that in sole NO_3^- nutrition, the reduced growth rates of herbaceous heathland plants were caused by N limitation. Yet, despite the increased growth of ammonium-fed *Arnica* plants, they did however develop stunted roots and yellow, chlorotic leaves. Especially the formation of stunted roots is commonly seen as a symptom of ammonium toxicity

(Mehrer & Mohr 1989). Ammonium containing nitrogen nutrition also led to an increased shoot/root ratio of *Arnica*: such an increase may have considerable effects on plants in natural habitats, as they become more sensitive to episodes of drought.

Aluminium, especially at low concentrations (50 µM), was beneficial to the growth of both species (Table 3, 4). Beneficial effects of (low) aluminium concentrations have been observed in more species, including *Arnica* (Hackett 1965, Pegtel 1987). Growth stimulating effects of aluminium are mainly observed in acid tolerant species and in species which accumulate aluminium (Marschner 1995). This aluminium accumulation is seen in both *Arnica* and *Cirsium* (Figure 1) and appears to be little influenced by nitrogen source. Yet, the nature of the stimulating effects of aluminium still remains unclear. It is thought that growth stimulation by aluminium is most probably a secondary effect, possibly due to alleviation of the toxic effects of other elements, for instance of Cu or P (Marschner 1995). Another possible explanation is the alleviation of H^+ toxicity by Al^{3+} (Marschner 1995).

Interactions between nitrogen source and aluminium on growth parameters were absent in *Arnica*, but were observed in shoot/root ratios and total and specific root length of *Cirsium* (Table 4). The changes in shoot/root ratio indicate a change in biomass allocation, whereas the decreases in total and specific root length strongly suggest a reduction in uptake capacity. Uptake capacity is partly determined by the total root surface (Marschner 1995); hence, by root length and by the thickness of roots. As both root length and thickness are severely reduced by aluminium and ammonium, especially in *Cirsium*, it is likely that the uptake capacity for water and nutrients is reduced.

Nutrient uptake is, however, not only related to physical factors as root length, but also by physiological processes. Uptake of nutrients in relation to nitrogen supply, in dependent upon the mineral nitrogen form taken up by the plants (Salsac *et al.* 1987, Pearson & Stewart 1993). Aluminium also interferes with the uptake of nutrients, especially of calcium, magnesium and phosphorus (Foy *et al.* 1988). It is thought that aluminium occupies cation-binding sites in the root-free space, thereby inhibiting cation uptake through competition or other mechanisms which are not yet fully understood (Rengel 1992). Furthermore, specific inhibition of calcium uptake by aluminium has been observed, probably by blocking the Ca-channels (Ryan *et al.* 1994) and nitrate uptake (Durieux *et al.* 1993, Calba & Jaillard 1997). In contrast, Klotz & Horst (1988) observed an increase in nitrate uptake per unit root length in soybean. They suggested that this increased uptake could compensate the loss in uptake capacity due to decreased root length caused by Al-toxicity.

In agreement with earlier observations on *Arnica* and *Cirsium* (De Graaf *et al.* 1997 1998), we observed severe reductions in calcium and magnesium concentrations, both affected by external aluminium and nitrogen nutrition (Table 5, 6). Potassium concentrations also decreased with ammonium nutrition, but (low) aluminium concentrations increased potassium uptake. As a result, indications that calcium, magnesium and potassium concentrations may become increasingly limiting were found in both species when supplied with ammonium and, in case of calcium and magnesium limitation, were also caused by aluminium (Figure 1). The increase in potassium uptake merely correlated with the increase in root length at low Al concentrations (Table 3, 4).

When assuming that data of natural grown plants in well developed populations give a good impression of a balanced nutritional status in these species (*Cirsium* (n=42):Hayati & Proctor 1990; *Arnica* (n=9): Pegtel 1994), comparison of our data with field data can elucidate actual nutrient limitations caused by experimental treatments. In general, calcium, magnesium and potassium concentrations of the plants grown without aluminium and with nitrate as the sole N source, equalled those of field grown plants, thus were assumed to be nutrionally balanced. The calcium concentrations of *Cirsium* and calcium and magnesium concentrations of *Arnica*, both treated with aluminium and ammonium, are far below those measured in field grown plants. However, when the shoot nutrients are expressed in terms of unit weight of nitrogen (Mg:N, Ca:N ratios), then the nutritional disharmony caused by ammonium and aluminium solutions becomes even more clear (data not shown). Now not only the severe reduction in calcium is far below field values, also the obtained magnesium concentrations in shoots of both species are severely reduced on aluminium and ammonium nutrition compared to the field values. Moreover, a serious potassium limitation due to ammonium nutrition becomes obvious in ammonium or mixed N-fed plants, although aluminium as such does not affect potassium concentrations. In *Arnica*, imbalances in potassium, calcium and magnesium are also indicated in plants grown with nitrate as a sole N source, as long as aluminium was present in the nutrient solution. Therefore, we think that limitations in calcium and magnesium may, at least partly, account for the observed growth reductions in *Cirsium* and *Arnica*. Potassium limitations in ammonium-fed plants may add to the other limitations, but reversely, improved K-uptake on low aluminium concentrations may, partially, account for the growth stimulation on low Al.

The results on plant phosphorus concentrations are unclear and vary between species and roots and shoots. In contrast to what is often found in coniferous trees (Boxman & Roelofs 1988, Bengtsson *et al.* 1994), phosphorus concentrations increased in nitrate-fed *Arnica* plants. In general, a decrease in phosphorus uptake is seen on nitrate nutrition, which is related to the competion for anion uptake. This is also observed in *Cirsium*, especially in the shoots. Aluminium (50 µM) increases phosphorus concentrations in *Arnica* and in the roots of *Cirsium*: this correlates with the growth stimulation by aluminium. However, shoot P concentrations in *Cirsium* are decreased by aluminium, but were always higher than in field grown plants (Hayati & Procor 1990, Pegtel 1994), which can be explained by the rather high P-concentrations in the nutrient solutions.

Cause for severe ammonium toxicity in Cirsium

Toxicity of ammonium was very obvious in *Cirsium* (Table 3). Ammonium-fed plants showed decreased growth of roots and shoots, and calcium, magnesium and potassium limitations were determined (Table 5, Figure 1). Moreover, although the nitrogen concentrations in roots and shoots increased in ammonium-fed plants, the nitrogen contents of both roots and shoots decreased (Table 5). This indicates ammonium toxicity in *Cirsium* (Figure 1). It is, however, unlikely that nitrogen itself is toxic to the plant and, to our knowledge, this has never been reported for higher plants. So, presumably, the nitrogen toxicity arises from processes associated with the uptake and/or assimilation of ammonium.

Dissociation of ammonium in the cell produces protons, which may lead to cytosolic acidification (Raven & Smith 1976). In order to avoid this cytosolic acidification, protons are excreted into the rhizosphere when ammonium is taken up (Raven & Smith 1976). In *Cirsium* however, excretion of protons following ammonium uptake hardly occurred (Figure 2). Moreover, bulk extracts of root material of plants grown on ammonium had a significantly lower pH than the bulk extracts of plants cultured with nitrate as a sole N source, indicating internal root acidification on ammonium nutrition (Figure 3). Gerendás and co-workers (1990) observed an acidification of the cytoplasmatic and vacuole pH in root tips of maize plants grown on ammonium at pH=4. They also showed that cytosolic and vacuolar pH were less affected in plants cultured on nitrate or on ammonium at higher pH. Hence, the authors hypothesised that the difference in internal pH between ammonium and nitrate treated plants at pH=4 was due to the effects of ammonium assimilation, since the energetics of H^+ transport out of the cell will become difficult as the external pH drops. This could lead to the observed decrease in pH in the cytosole and vacuoles (Gerendás *et al.* 1990). We think that a similar inability of *Cirsium* to excrete protons against a external pH gradient is, besides the severe cation limitations, probably a major cause for the severe ammonium toxicity observed in this and a previous study (De Graaf *et al.* 1998).

In the introduction we posed ourselves a few questions: whether the effects of aluminium were altered by N source and whether ammonium toxicity was ameliorated by aluminium. Both questions can be answered positively, especially when looking at processes at the plant nutritional status. The beneficial effects of low aluminium concentrations on plant dry weight partly reduced the toxic effects of ammonium. This may be due to enhanced uptake of phosphorus in aluminium treated plants. However, the beneficial effects of low aluminium in *Cirsium* do not compensate for the negative effects of ammonium: they are probably caused by a reduction in root growth, by internal acidification and by a severe reduction in calcium, magnesium and potassium uptake. Although ammonium did not excert negative effects on the growth of *Arnica*, it was observed that plants show some of the ammonium toxicity symptoms and also show severely decreased calcium, magnesium and potassium concentrations. It is well possible that these phenomena become more dominant with increasing ammonium concentration. In a previous study (De Graaf *et al.*

1998), it was shown that higher ammonium concentrations in the nutrient solutions (500, 1000 µM) resulted in a reduction in seedling survival of *Arnica* and in reduced growth. It is clear that despite the fact that both species occur in habitats with pH 4.5-6, *Cirsium* and *Arnica* differ in their response to aluminium and nitrogen nutrition.

Differences in aluminium tolerance in Arnica

The response of *Arnica* to aluminium in this experiment, with nitrate as the sole nitrogen source, differed substantially from that of *Arnica* seedlings in earlier studies (De Graaf *et al.* 1997), but was in agreement with the results of Pegtel *et al.* (1987). Instead of a gradual decrease in root and shoot biomass with increasing aluminium concentration in the nutrient solution, biomass production increased at an external concentration of 50 uM aluminium in the present study. The difference in the response to aluminium can be explained for two reasons. Firstly, the seeds used in the experiments originated from different populations and were collected in different years. The seeds used in this experiment originated from a population which grew on rather acidic soils (pH 4.5 – 5.0), whereas the seeds used in earlier experiments (De Graaf *et al.* 1997) were supplied by a commercial grower. It is likely that those plants were grown on less acidic soils. As environmental conditions, e.g. soil acidity, may play an important selecting role on plants and thus on populations, the offspring of the population used in this study may have been more aluminium tolerant than that of the population used in the former study by De Graaf *et al.* (1997).

Secondly, differences in seedling age may also explain the difference in aluminium toxicity observed in *Arnica*. The seedling phase is often the most sensitive phase in the plants life (Fenner 1987). As seedlings age, they may become more tolerant to environmental factors. The *Arnica* seedlings used in this experiment were about two weeks older than in our previous study (De Graaf *et al.* 1997) and might therefore be less sensitive to aluminium. The uncertainty as to what extent differences in aluminium tolerance of *Arnica* are caused by either plant age or population, emphasises the need for more research.

Ecological implications

Aluminium toxicity in acid soils is generally restricted to soils with pH<4.5, as phytotoxic Al^{3+} concentrations are only found below these pH values (Kinraide 1997). Therefore, acidification of the rhizosphere induced by ammonium uptake may affect the extent of aluminium toxicity. In contrast, aluminium toxicity may be reduced by nitrate uptake, as rhizosphere pH will be increased by nitrate uptake. Although it is now known that there is no causal relationship between plant-induced pH increases after nitrate uptake and aluminium tolerance (Taylor 1988, Myasaka *et al.* 1989, Calba & Jaillard 1997), it is likely that detoxification of Al^{3+} by OH^- or phenolic acids, may play an important role in natural habitats. Contrastingly, the decrease in rhizosphere pH after ammonium uptake may also be of importance in natural habitats.

Despite the predominant uptake of ammonium from mixed N sources in the studied species, it is possible that they also take up nitrate in soils. In soils, ammonium is a rather immobile nutrient, and as uptake proceeds it is likely that depletion zones will develop in the rhizosphere (Marschner *et al.* 1991). With an increasing proportion of nitrate available in the rhizosphere, plants will begin to take up nitrate as N source, concommittantly reducing the negative aspects of imbalanced N nutrition.

However, when ammonium concentrations in the soil increase, for instance as a result of fertilisation, atmospheric N input or inhibition of nitrification due to soil acidification, nitrogen nutrition may become imbalanced. The uptake of larger amounts of ammonium may cause a further acidification of the rhizosphere, thereby further inhibiting nitrification, which will result in an increase in the NH_4^+/NO_3^--ratio. Soil acidification due to ammonium uptake is not only hypothetical: Arnold & Van Diest (1991) calculated that in *Larix kaempferi*-forests, soil acidification due to ammonium uptake might be as high as 3.04 kM H^+ ha^{-1} y^{-1}.

Moreover, acidification of the rhizosphere may enhance cation leaching. This study has shown that limitations of calcium, magnesium and potassium are major causes of the reduced performance of *Arnica* and *Cirsium* on ammonium nutrition, or when external aluminium concentrations solutions increase. Leaching of calcium, magnesium and potassium from upper soil layers will decrease the availability of these nutrients, thereby increasing the risk of nutrient limitations.

When soil pH falls below 4.5, the concentration of Al^{3+} in the rhizosphere will increase. And although low (50 µM) aluminium concentrations have a stimulating effect on *Cirsium* and *Arnica*, higher (200 µM) aluminium concentrations are toxic (De Graaf *et al.* 1997). Furthermore, the beneficial effects of calcium on aluminium toxicity, which have been shown by many authors (e.g. Ryan *et al.* 1994, De Graaf *et al.* 1997), will be reduced and aluminium toxicity may become more pronounced.

Therefore, we conclude that soil acidification is a serious threat to *Arnica* and *Cirsium*, despite the fact that low aluminium concentrations are beneficial to these species. *Cirsium* is extremely sensitive to a combination of ammonium nutrition and low pH. In both species the uptake of calcium and magnesium is severely inhibited by a combination of ammonium nutrition and aluminium in the nutrient solution. As plants continue to use ammonium as the major nitrogen source, a potential risk of an ongoing acidification process exists, which is maintained by the plants themselves.

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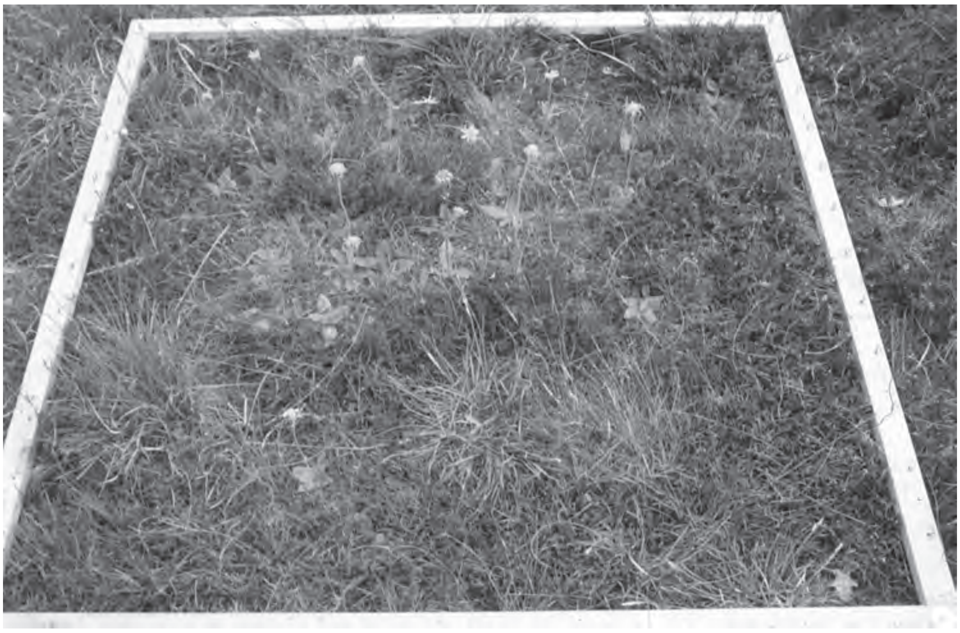
CHAPTER 6

RESTORATION OF SPECIES-RICH DRY HEATHS: THE IMPORTANCE OF APPROPRIATE SOIL CONDITIONS



WITH PETER J.M. VERBEEK, ROLAND BOBBINK & JAN G.M. ROELOFS

RESTORATION OF SPECIES-RICH DRY HEATHS: THE IMPORTANCE OF APPROPRIATE SOIL CONDITIONS

SUMMARY

The aim of many rehabilitation projects in degraded ecosystems is to restore biodiversity. In order to achieve this, restoration often focusses on abiotic conditions as they are the main cause for the degradation of the ecosystem. The necessity of restoring the soil conditions for rehabilitation of heathland vegetation is shown by this study.

Three dry heathland areas were studied: an acidic species-poor heath which is degrading as a result of atmospheric nitrogen deposition, an acidified matgrass sward and an abandoned grassland. We aimed to restore the characteristic plant communities of the three heathlands: a CALLUNO-GENISTION PILOSAE community in the acidic species-poor heath and a NARDO-GALION SAXATILIS community in the acidified matgrass sward and abandoned grassland. Restoration methods included sod cutting to the mineral soil layer, liming and a combination of sod cutting and liming. Effects of methods on top soil chemistry, vegetation development and development and demography of the rare *Arnica montana* are shown and discussed. Furthermore, we discussed the importance of seed availability in relation to the importance of restoring soil conditions for successful rehabilitation of heaths.

INTRODUCTION

Degraded ecosystems are restored for many reasons, varying from conservation of rare species and biodiversity to the protection of human health in seriously polluted areas. In all cases, restoration of degraded ecosystems should begin with counteracting the main causes for the decline. However, some ecosystems are affected by a deteriorating factor to such an extent, that removal of the cause for decline is insufficient for ecosystem rehabilitation (Hobbs & Norton 1996). For example, soil acidification has a great impact on soil chemistry, but the effects of acidification are easily reversed by the addition of lime. However, as a result of acidification, characteristic plant species of an ecosystem may have disappeared. Their return does not only depend on the habitats' quality, e.g. the appropriate soil conditions, but also depends on the capacity of the species to recolonise the habitat. This paper presents data from a restoration experiment, carried out in acidified and eutrophied heathland vegetation, focussing on the question whether dry heath vegetation will recover after restoring the soil conditions.

In the Dutch lowland heathland landscape, different plant communities can be found. Most of the drier parts of the landscape were originally rather poor in phanerogamic species and dominated by the dwarf shrub *Calluna vulgaris* (CALLUNO-GENISTION PILOSAE). In other parts, more species-rich matgrass sward communities were found (NARDO-GALION SAXATILIS), of which many characteristic species are currently rare. Both communities have declined since the beginning of this century, due to changes in land use and atmospheric input of NH_y and SO_x. These atmospheric inputs led to increased nitrogen availability and soil acidification. The dwarf shrub dominated heath vegetations are very sensitive to increased nitrogen availability: grasses such as *Molinia caerulea* and *Deschampsia flexuosa* benefit more than the heather species from the increased nitrogen availability, and so, eventually the heathlands are changed into grasslands (e.g. Bobbink *et al.* 1992, Aerts & Heil 1993). It is not unlikely that a similar process takes place in the species-rich heaths, since the competitive abilities of the NARDO-GALION SAXATILIS species *Arnica montana* and *Viola canina* in comparison to the grass *Agrostis canina* are strongly reduced by NH_3input (Dueck & Elderson 1992). Moreover, soil acidification seriously affects many characteristic species of the NARDO-GALION SAXATILIS communities (Van Dam *et al*. 1986, Fennema 1992, Roelofs *et al.* 1996). Experiments with *Arnica montana* have shown that especially high ammonium and aluminium concentrations are toxic to this species at low pH (Heijne 1995, De Graaf *et al.* 1997, De Graaf *et al.* 1998). In contrast, the CALLUNO-GENISTION PILOSAE species occur on more acid soils and are not affected by soil acidification (Houdijk *et al*. 1994, De Graaf *et al.* 1997).

As dry heath and matgrass swards are mainly threatened by nitrogen eutrophication and soil acidification, restoration should focus primarily on counteracting the negative consequences of these processes, as well as on the reduction of the deposition of atmospheric N and S compounds. Removal of accumulated nitrogen can be achieved by sod cutting (‘plaggen’), which removes the above ground parts of the vegetation and the organic soil layers (Heil & Aerts 1993, Diemont 1994). In the past, sod cutting of Western Europe heaths was part of a widespread agricultural system, but due to the introduction of artificial fertilisers this practice was abandoned (Gimingham & De Smidt 1983). It has since been reintroduced as an adequate tool for restoration of *Calluna vulgaris* dominated heath (Helsper *et al.* 1983, Werger *et al.* 1985). As well as reducing soil nutrients, the bare soil which results from sod cutting offers suitable germination conditions to many species (Miles, 1973). However, sod cutting does have a serious drawback: since heathland species have a the greatest proportion of their seeds stored in the upper centimetres of the soil (Bruggink 1993, Putwain & Gillham 1990), a substantial part of the seedbank is removed by sod cutting. This may restrict the reestablishment of species, especially those with a short-lived seedbank of which all seed is probably present in the upper soil layers. Many NARDO-GALION SAXATILIS species have such short-lived seed banks (Bakker *et al.* 1996).

Liming of the top soil is a method widely used in agriculture and forestry to ameliorate soil acidification. In recent decades it has also been used in nature conservation in order to reduce the negative effects of acidic atmospheric deposition in forests and lakes (Laudelout 1991, Kreutzer 1995, Henrikson & Broden 1995). In general, pH and base cation concentrations of the soil are increased whereas aluminium concentrations are decreased by applications of lime (Kreutzer 1995). Reports on the effects of liming on heathland species are limited and vary from beneficial (Wilson 1995) to detrimental (Rodenkirchen 1995).

We studied the effects of sod cutting, liming and a combined treatment on dry heath, matgrass sward and abandoned grassland in a Dutch heathland nature reserve. The effects on soil chemistry and vegetation were studied from 1990 to 1996, in order to quantify the restoration of the affected plant communities. Special attention has been paid to the rare *Arnica montana* and its response to the different management regimes. The main aim of the experimental management was to regenerate appropriate soil conditions for a CALLUNO-GENISTION PILOSAE community in the dry heath and a NARDO-GALION SAXATILIS community on the matgrass sward and abandoned grassland. The dry heath and the matgrass sward showed signs of decline at the onset of the experiment, probably due to nitrogen enrichment and soil acidification, respectively. We hypothesised that these negative signs would intensify without restoration practices. Furthermore, it was our hypothesis that sod cutting would reduce the soil nutrient contents and that both the dwarf-shrub dominated heath and the matgrass sward would benefit from it. Thirdly, we expected to see a reversal of soil acidification under all vegetation types, notwithstanding the fact that we used low doses of lime (150 - 600 kg ha^{-1}) which aimed only at small increases in pH and base cation concentrations. We hypothesised that these changes in soil chemistry would be benificial in particular to the matgrass sward vegetation, since these species are sensitive to acidification, whereas the characteristic species of the CALLUNO-GENISTION PILOSAE communities are adapted to acidic soils and would not have been severely affected by acidification. The abandoned grassland was included in the experiment to examine whether sod cutting could accelerate the vegetational transition to a NARDO-GALION SAXATILIS community.

Nomenclature of plant species and plant communities follows Van der Meijden (1990) and Schaminée *et al.* (1996), respectively.

MATERIALS AND METHODS

Site description

The experiments were carried out in ‘de Schaopedobbe’, a Dutch nature reserve (52° 57’N, 6°16’E), owned and managed by ‘ It Fryske Gea’. Dry, inland heath (CALLUNO-GENISTION PILOSAE communities) and matgrass swards (NARDO-GALION SAXATILIS communities) form a large part of this reserve. These communities are found on sandy soils of Pleistocene origin, in which a podzollic profile developed. Three parts of the nature reserve, with a vegetation as homogenous as possible, were selected for the experiment: a matgrass sward (NARDO-GALION SAXATILIS community) in which the original *Arnica montana* population was declining (referred to as ‘Matgrass sward’). This decline was probably caused by soil acidification and/or nitrogen eutrophication.

The vegetation of the second part ('Heath') could be described as CALLUNO-GENISTION pilosae, and lacked *Arnica montana* plants. The third study area was an abandoned grassland, with a recently expanding *Arnica montana* population ('Grassland'). In contrast to the other parts, the Grassland was used for arable agriculture, and had received fertilisers and lime in the past. After abandonment, a grassland management regime was initiated in order to develop a species-rich grassland vegetation. At the beginning of the experiment (March 1990), the pH of the top soil layer (10 cm) varied between 3.8 and 4.3 in the Heath, between 4.1 and 4.7 in the Matgrass sward and between 4.2 and 5.1 in the Grassland.

During the experimental period, all sites were mown anually in August, with the hay being subsequently removed. This was common practice before in the Matgrass sward and Grassland, but was first carried out in 1990 in the Heath.

Experimental design

Two plots (30 x 10 m) were laid out in each of the studied sites, in March 1990. In one plot per site, the top soil layer, including above-ground parts of the vegetation, was removed by sod cutting to the mineral soil layer. In both the sod cut and non-sod cut plots, subplots (5 x 10 m) were treated with lime (chalk, 100% $CaCO_3$, grain diameter 0.1-10 mm) or dolomite (80% $CaCO_3$, 20% $MgCO_3$, grain diameter < 0.1 mm). In order to increase the base saturation of the top soil layer to 80 %, 150 kg ha^{-1} lime (limestone or dolomite) was applied to the Grassland subplots, 300 kg ha^{-1} to the Matgrass sward subplots and 600 kg ha^{-1} to the Heath subplots. Lime applications were repeated in July 1991. In each plot, two control subplots were not limed; a 5 m wide zone divided the limed and unlimed.

The effects of sod cutting and liming on top soil chemistry (10 cm) were studied twice a year from March 1990 to August 1995. Soil samples were taken with an auger (diameter 3 cm, 8 subsamples per subplot); subsamples were mixed and stored in polyethylene bags at 4°C until analysis. In all samples, waterextractable pH, Al, Ca, Al/Ca-ratio, NO_3^- and P were measured, as were exchangeable Ca, Mg, K and NH_4^+. Total mineral nitrogen concentrations (N_{min}) are the sum of waterextractable NO_3^- and exchangeable NH_4^+. In addition, soil pH was determined every two months during the first year of the experiment. The soil organic matter content was determined immediately in March 1990, by weight loss after ignition (550 °C, 4 h).

The vegetation of all subplots was described in the summers of 1990, 1992, 1994 and 1995, using the Braun-Blanquet approach (Westhoff & Van der Maarel, 1978). As vegetation development was slow, only the data of 1990 (non-sod cut subplots) and 1995 (all subplots) are presented. More specific information on the effects of liming on the performance of established *Arnica montana* populations in the Matgrass sward was gained by counting the numbers of rosettes and flower buds of the plants in the non-sod cut subplots. These observations were made at the end June of 1991, 1994, 1995 and 1996. We were unable to study the effects of liming on established *Arnica montana* populations in the Grassland, due to the fact that these were only present in the control subplots. No established *Arnica montana* plants were present in the Heath.

In the sod cut plots of the Matgrass sward, an experiment was set up to study the effects of liming on the demography of *Arnica montana* in detail. A hundred seeds were sown in a grid (0.5 cm below the soil surface, 10 cm distance between seeds) in July 1991. The seeds of *Arnica montana* were collected two weeks earlier in the direct neighbourhood of the experimental plots; 98% of the sampled seeds germinated under laboratory conditions (20 °C). Germination of the seeds and survival of the seedlings and plants were regulary noted until June 1996. From 1994 onwards biomass was estimated annually with a non-destructive method which consisted of multiplication of the maximum leaf length with the maximum leaf width and the number of leaves. This method proved to be a good estimation of biomass of *Arnica montana* ($R=0.77$, $p<0.001$, greenhouse conditions). An identical experiment was carried out in 1992 in the control and dolomite treated plots of the Heath; as in the Matgrass sward, biomass estimations on *Arnica montana* began three years after sowing.

Extraction methods and chemical analyses

70 g of thoroughly mixed fresh soil was mixed with 200 ml bi-distilled water (for determination of waterextractable elements) or 200 ml 0.2 M NaCl solution (for determination of exchangeable elements). The mixtures were shaken for one hour (120 movements min^{-1}), after which pH of the solution was measured (Radiometer type PHM 82 pH-meter). Hereafter, the solution was centrifuged (12000 rpm, 20 min) and the supernatant was stored in polyethylene bottles at -28° C. Al, Ca, Mg and P concentrations were measured using an ICP (type IL Plasma 200), NO_3^- and NH_4^+ concentrations were determined colorimetrically with a continous-flow autoanalyser (Technicon AAII system) and K concentrations were quantified with flame photometry (Technicon Flame photometer IV).

Statistical analyses

To determine the effects of sod cutting, an analysis of variance (GLM procedure, SAS 6.0) was performed on soil data; data were log-transformed in order to fit a normal distribution. The effects of liming on soil chemistry in the separate plots were tested with a regression analysis (GLM procedure, SAS 6.0); a separate slope model was used in order to detect differential time effects between treatments.

Germination and survival of sown *Arnica montana* plants were tested with a PHREG procedure (SAS 6.0). This is a regression analysis of survival data based on the Cox proportional hazards model. Three years after sowing and onwards, the effects of liming on the estimated biomass of the living *Arnica montana* plants were tested with an analysis of variance (GLM procedure, SAS 6.0) following log transformation.

RESULTS

Effects on soil chemistry

SOD CUTTING

Sod cutting instantly reduced the concentrations of most nutrients and organic matter content in the top soil layer (Table 1). P concentrations were strongly reduced, and not detectable in the sod cut plots of the Heath and Matgrass sward. The mineral nitrogen concentrations decreased with 40% compared to the intact vegetation. The top-soil pH of the Heath increased to 4.3, whereas in the non-sod cut parts of the Heath pH remained at 4.1. In the Matgrass sward and the Grassland, pH was not affected by sod cutting.

During the five years following sod cutting, the concentrations of most nutrients in the sod cut plots remained below those of the concentrations in the soils under intact vegetations, although some nutrients showed considerable fluctuations (Figure 2 & 3, data for P not shown). However, mineral nitrogen concentrations increased considerably to 1500-3000 µmol kg^{-1} dry soil during the first year after sod cutting (Figure 1), compared to 500-1000 µmol kg^{-1} dry soil in the non-sod cut plots. Both ammonium and nitrate concentrations were enhanced by sod cutting (Table 2), but the increase in ammonium concentrations was especially high in the Heath and

Table 1 Soil characteristics (geometrical means) with (+) and without (-) sod cutting of unlimed plots, two weeks after sod cutting (March 1990). pH and P are determined in waterextracts, Ca+Mg+K in 0.2 M NaCl extract. $N_{mineral}$ is the sum of waterextractable NO_3^- and NaCl-extractable NH_4^+. All nutrients are expressed in µmol kg^{-1} dry soil, except Ca+Mg+K, which is in µeq kg^{-1} dry soil.
Significance: ns: p>0.1, *:p<0.05, **: 0.01<p<0.05, ***: p<0.01. If 0.1<p<0.05 then the p-value is indicated. N=4.

	Heath			**Matgrass sward**			**Grassland**		
Sod cutting	-	+		-	+		-	+	
pH	4.01	4.31	*	4.48	4.43	ns	4.65	4.64	ns
Al	93	82	ns	41	130	ns	110	72	**
Ca_{exch}	2015	544	***	1656	760	*	2475	925	**
Mg_{exch}	683	564	***	695	594	*	691	570	**
Base cations	6351	1978	***	6246	2812	*	7681	2762	**
NO_3^-	3.3	47.1	0.09	137.5	73.6	ns	69.6	51.2	ns
$NH_{4\ exch}^+$	713	422	ns	502	305	ns	517	268	**
$N_{mineral}$	808	482	ns	647	387	ns	639	322	*
P	8.5	0.0	***	6.4	0.0	*	18.0	8.0	*
% org. matter	17.5	7.0	***	14.1	11.8	*	7.6	5.5	ns

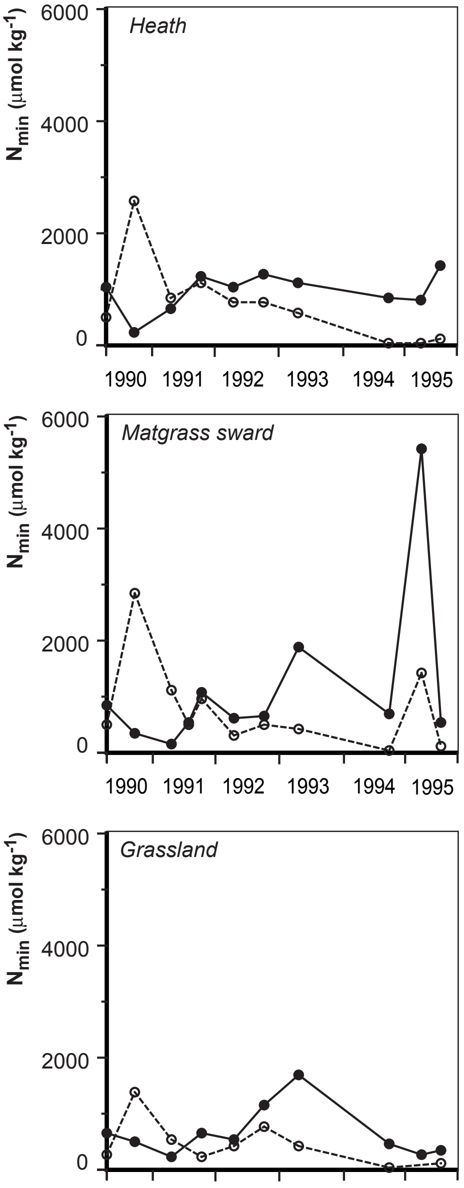


Figure 1 The effects of sod cutting on mineral nitrogen concentration ($N_{mineral}$) in the unlimed plots. Closed circles, solid line: non sod-cut plots, open circles, dashed line: sod-cut plots.

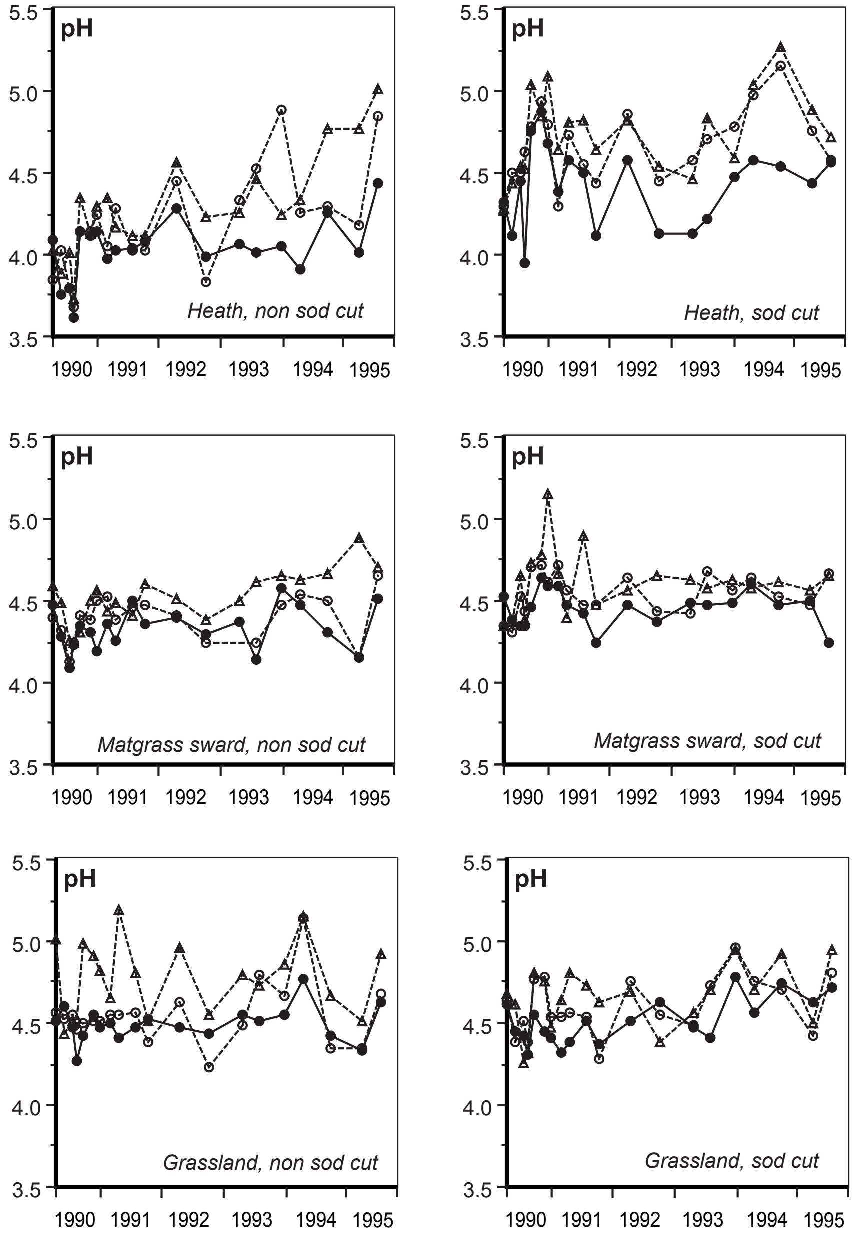


Figure 2 The effects of sod cutting and liming on top soil pH. Closed circles: control (unlimed) subplots; open circles: limestone treated subplots; open triangles: dolomite treated subplots.

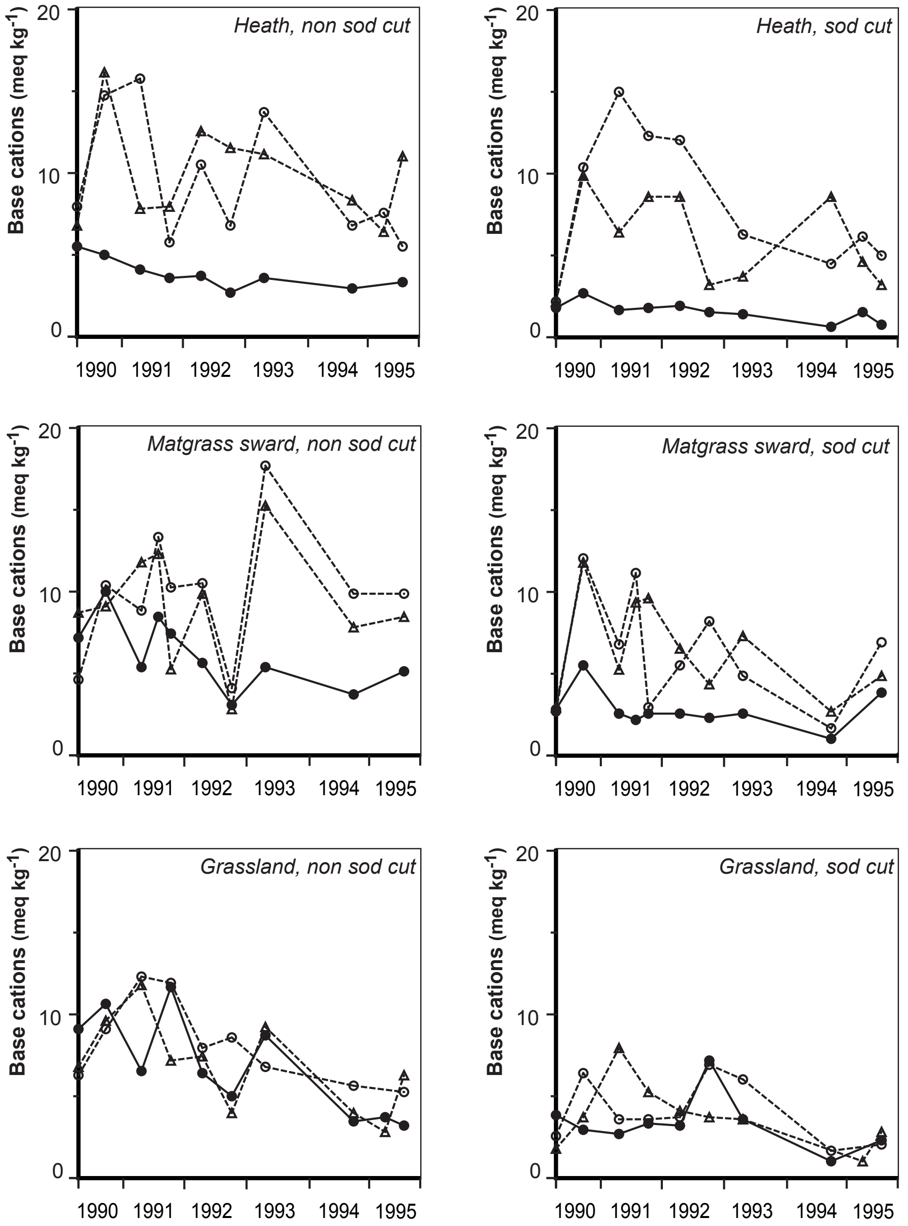


Figure 3 The effects of sod cutting and liming on base cation concentrations (Ca+Mg+K, in meq kg^{-1} dry soil). Closed circles: control (unlimed) subplots; open circles: limestone treated subplots; open triangles: dolomite treated subplots.

Table 2 Peak values in exchangeable NH_4^+ and waterextractable NO_3^- concentrations, the ratio between NH_4^+ and NO_3^- and total mineral nitrogen concentrations ($N_{mineral}$) in the first year after sod cutting (unlimed subplots, N=2). Concentrations in µmol kg^{-1} dry soil. *: N=1. -: not sod cut; +: sod cut.

	NH_4^+		NO_3^-		NH_4^+/NO_3^-		$N_{mineral}$	
Sod cutting	-	+	-	+	-	+	-	+
Heath	186	2426	12	135	4.85*	47.4	198	2562
Matgrass sward	310	2520	25	252	16.9	16.7	335	2772
Grassland	383	929	110	761	4.6	14.3	493	1691

Matgrass sward (up to ca. 2520 µmol kg^{-1} dry soil). In all sites the NH_4^+/NO_3^--ratio was in the same order of magnitude in the sod cut and non-sod cut plots. One year after sod cutting, mineral nitrogen concentrations reverted to the initial values after sod cutting and remained low for the rest of the experimental period (< 100 µmol kg^{-1}), except in the Matgrass sward, where a second peak in $N_{mineral}$ was measured after the winter of 1995.

LIMING

The effects of liming (treatments with dolomite or limestone) are presented as comparisons between limed and control (unlimed) plots, within the same vegetation type, with or without sod cutting. The effects of liming were, generally, most pronounced in the Heath, followed by the Matgrass sward. In the Grassland, hardly any significant effects of liming on top soil chemistry were found (Table 3). Liming affected soil chemistry more in sod cut plots than in plots with vegetation cover. The two liming materials, dolomite and limestone, affected soil chemistry in similar ways. Therefore, they will be discussed together, except where the results differed considerably between dolomite and limestone treatments.

The response of soil factors related to the buffering capacity to liming was rapid (Figures 2 & 3): pH and base cation concentrations increased almost immediately following the addition of limestone or dolomite. Despite considerable variation in top soil pH within the subplots, liming treatments increased the pH in all subplots (Figure 2). This increase was often significant (Table 3). Liming increased the pH of the sod cut subplots to similar values, regardless of vegetation type (median pH: 4.7 ± 0.1 in limed subplots; 4.5 ± 0.1 in unlimed subplots), whereas the initial differences in pH between areas were still noticeable after liming in the non-sod cut plots (median pH: 4.2, 4.5 and 4.6 in limed and 4.0, 4.3 and 4.5 in unlimed Heath, Matgrass sward and Grassland subplots, respectively; differences in median pH between dolomite and limestone treatments did not exceed 0.1 pH value).

Table 3 Changes in soil chemistry after liming during the period 1990 – 1995. All liming treatments have been compared to the control. ++: increase, $p<0.05$; +: increase, $0.05<p<0.25$; =: no difference; -: decrease, $0.05<p<0.25$; --: decrease, $p<0.05$. Al/Ca ratio is based on waterextractable Al and Ca; Ca_{ex}: exchangeable Ca; Mg_{ex}: exchangeable Mg; base cations: sum of 2^*Ca_{ex}, 2^*Mg_{ex} and K_{ex}. $N_{mineral}$: mineral nitrogen. L: limestone; D: dolomite.

	Heath				Matgrass sward				Grassland			
	Not sod cut		Sod cut		Not sod cut		Sod cut		Not sod cut		Sod cut	
Liming	L	D	L	D	L	D	L	D	L	D	L	D
pH	=	=	+	++	=	+	=	++	++	=	++	+
Al	=	-	=	=	=	-	=	+	=	=	=	=
Al/Ca	--	--	--	--	=	=	=	--	+	=	=	=
Ca_{ex}	++	++	++	++	=	=	++	++	=	=	+	+
Mg_{ex}	+	++	++	++	=	=	=	++	=	=	=	++
Base cations	++	++	++	++	=	=	++	++	=	=	=	++
$N_{mineral}$	=	=	-	-	=	=	=	=	=	=	=	-
P	=	=	--	--	=	=	=	=	=	=	=	=

Liming significantly increased base cation concentrations in all Heath subplots, in the sod cut subplots of the Matgrass sward and only in the dolomite treated, sod cut subplot of the Grassland (Table 3, Figure 3). The increase was mainly due to an increase in exchangeable calcium concentration; magnesium concentrations increased only in the dolomite-treated subplots and in most limed Heath subplots (Table 3). Towards the end of the experiment, base cation concentrations tended to decrease in all limed subplots. This decrease was significant in the limestone treatment in both the Heath vegetation (non-sod cut) and the dolomite treatment in the sod cut Matgrass sward.

No significant effects of liming treatments on aluminium concentrations were observed, but there was a significant reduction in Al/Ca-ratios in all limed Heath subplots and in the dolomite-treated, sod cut part of the Matgrass sward (Table 3). In general, median Al/Ca values varied over the period after liming from 1.2-3.8 (limed Heath subplots), 2.0-3.5 (limed Matgrass sward subplots) and 2.2-4.7 (limed Grassland subplots), whereas they varied in the unlimed subplots from 3.9-6.9 (Heath, with and without sod cutting respectively) 5.2-7.6 (Matgrass sward, with and without sod cutting, respectively) and 3.1-3.2 (Grassland, with and without sod cutting resp.). In the limed plots, Al/Ca-values were more reduced in the sod cut parts of the Heath and Matgrass sward than in the parts of the plots with vegetation present. In the Grassland, the opposite was observed, with even a slight increase in median Al/Ca-ratios in the plots covered with vegetation.

In contrast, mineral nitrogen and phosphorus concentrations showed little response to liming, regardless of whether sods were removed or not. A significant reduction in phosphorus concentrations was found only in the limed, sod cut Heath subplots. A few remarkable trends were, however, observed in the mineral nitrogen concentrations (data not shown). Firstly, during the relatively warm and wet winters of 1994 and 1995 nitrate concentrations more than doubled in the limed plots of the sod cut Grassland and of the non-sod cut Matgrass sward, in comparison to the concentrations in the control plots of those sites. During the summers following these winters, nitrate concentrations were lowered to concentrations similar to those in the controls. Secondly, the peak concentrations for ammonium that were measured in unlimed sod cut subplots during the first year following sod cutting (Figure 1) were reduced by 10-25% by the liming treatment. After the first year, ammonium and nitrate concentrations did not differ anymore between the limed and unlimed plots, nor was there any difference due to liming in the soils beneath the intact vegetations.

Effects on the vegetation

The species composition of non-treated vegetations changed little during the six-year experimental period, although species' number increased slightly in most subplots (Table 4; non-sod cut, control plots). Furthermore, vegetation cover of the non-sod cut parts of the Heath decreased by 20-25%, probably a consequence of the introduced mowing regime. In one of the control plots, the reduction in cover could be ascribed almost exclusively to the decrease in cover of the dwarf shrub *Empetrum nigrum*, whereas in the other control plot, *Festuca ovina* declined considerably.

A trend towards a greater abundance of some grasses was observed. In particular *Agrostis canina* increased its cover in both the Grassland and the Matgrass sward, whereas *Deschampsia flexuosa* and *Molinia caerulea* increased in the Heath. The latter species also increased considerably in one of the control plots in both the Grassland and Matgrass sward.

SOD CUTTING

Five years after sod cutting had created a bare soil surface, the vegetation cover of the Matgrass sward and Grassland equalled that of the parts that were non-sod cut (80-90 % in the sod cut and 80-95% in the non-sod cut parts; Table 4). In the sod cut Heath vegetation, the canopy was not yet closed in 1995 (50-70% cover). Almost all plant species present in the non-sod cut plots in 1990, had returned in 1995. In most sod cut plots species number, was higher in 1995 than in the plots that had not been sod cut. The dwarf shrub *Calluna vulgaris* benefited especially from sod cutting: in all sod cut areas this species became more abundant than on the non-sod cut plots. In the Heath, *Calluna vulgaris* had become the dominant species at the

Table 4 Vegetation the Heath (Table 4A), Matgrass sward (table 4B) and Grassland (table 4C) in 1990 and 1995. C1: control1 (unlimed); C2: control2 (unlimed); L: limestone; D: dolomite. -: non sod-cut subplot; +: sod-cut subplot. * Characteristic species of the NARDO-GALION SAXATILIS; #: Characteristic species of the CALLUNA-GENISTION PILOSAE. [1]: subspecies *congesta.*

4A Heath

Experimental plot	**C1**	**C1**	**C1**	**C2**	**C2**	**C2**	**L**	**L**	**L**	**D**	**D**	**D**
Year	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**
Sod cut	-	-	+	-	-	+	-	-	+	-	-	+
Vegetation cover (%)	90	55	70	90	70	50	80	80	60	85	85	55
Number of species	13	15	21	14	15	17	11	17	24	12	15	21
Matgrass sward species												
Agrostis canina	1	+	2m	1	+	2a	1	2a	2a	1	2m	2a
Agrostis capillaris			r			+			+			
Arnica montana *			+						+			r
Danthonia decumbens *	1	+	1	1	+	+	+	+	1	1	+	+
Galium saxatile *	1	1	+	2m	1	+	2m	2a	+	2m	2m	1
Hieracium pilosella			+		r			+	1		+	1
Nardus stricta *	r	1		+	+	+	+	1		+	+	
Potentilla erecta *			+			r		+	+			+
Veronica officinalis *												r
Heathland species												
Calluna vulgaris #	2a	2a	4	2a	2b	3	2a	2a	3	2b	2b	3
Empetrum nigrum	4	2a		2a	2a	r	2m	+		+	+	
Erica tetralix									+			+
Genista anglica #	+	+	+	r	r							
Genista pilosa #			r			r		r	+			r
Frequently found species in matgrass swards and heathland vegetations												
Carex pilulifera	+	2m	2m	2m	2m	2a	1	2m	2a	2m	2m	2a
Deschampsia flexuosa	r	2a	+		+	+	3	2a	+	+	1	r
Festuca ovina	2a	2b	2m	3	2a	1	3	3	2a	3	3	2a
Molinia caerulea	+	2a	1	3	3	1	2a	2a	1	2b	2a	+
Rumex acetosella		1	+	r	1	1	2m	2m	+	+	2m	+
Remaining species												
Betula pubescens									r			
Carex panicea												+
Cerastium semidecandrum								+			+	
Hieracium laevigatum			r						+			+
Hieracium umbellatum			r						r			

Continued on page 122

Table 4 Continued

4A Heath Experimental plot	C1	C1	C1	C2	C2	C2	L	L	L	D	D	D
Year	1990	1995	1995	1990	1995	1995	1990	1995	1995	1990	1995	1995
Sod cut	-	-	+	-	-	+	-	-	+	-	-	+
Hieracium vulgatum									+			r
Hypochaeris radicata		+	1	r	+	1		2m	2m	r	2a	2m
Plantago lanceolata								r				
Prunus serotina		r	r			r			r			r
Quercus robur	r											
Rhamnus frangula			r	r	r				r		r	
Sorbus aucuparia	r	r	r	+					r			r
Spergula morisonii						+						
Taraxacum officinale s.s.									r			

4B Matgrass sward Experimental plot	C 1	C 1	C1	C 2	C 2	C 2	L	L	L	D	D	D
Year	1990	1995	1995	1990	1995	1995	1990	1995	1995	1990	1995	1995
Sod cut	-	-	+	-	-	+	-	-	+	-	-	+
Vegetation cover (%)	90	80	75	90	80	80	95	90	80	95	80	85
Number of species	17	22	20	15	20	20	14	24	25	14	26	23
Matgrass sward species												
Agrostis canina	2m	2b	2a	2a	2a	2b	2a	2b	2b	2a	2b	2a
Agrostis capillaris		1	+			+		+	+			+
Arnica montana *	1	1		1	1	r	1	1	+	1	1	r
Danthonia decumbens *	1	1	2a	2a	2a	2a	2a	1	1	2a	2a	+
Euphrasia stricta											r	
Galium saxatile *	2b	2a	2m	2b	2m	2m	2b	2m	2m	2b	2m	2m
Hieracium pilosella		r	1					+	2m		1	2m
Nardus stricta *			r	+	+			+			2a	
Polygala serpyllifolia						+		+	1		1	1
Potentilla erecta *	+	+	1	r		1		+	2a		+	2m
Heathland species												
Calluna vulgaris #	2a	2a	3	2a	2a	3	2a	1	3	2a	1	4
Erica tetralix	r	+			r						r	
Genista anglica #	+	+	2m	+	+	1	r	+	2m	1	+	2m
Genista pilosa #									r			

4B Matgrass sward

Experimental plot	**C 1**	**C 1**	**C1**	**C 2**	**C 2**	**C 2**	**L**	**L**	**L**	**D**	**D**	**D**
Year	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**
Sod cut	-	-	+	-	-	+	-	-	+	-	-	+
Frequently found species in matgrass swards and heathland vegetations												
Carex pilulifera	2b	2a	2a	2a	2m	2a	2b	2a	2a	2a	2a	2a
Deschampsia flexuosa		r			r							
Festuca ovina	3	2a	2m	3	2a	2m	3	2b	2m	3	2b	2a
Molinia caerulea	2b	3	1	3	3	2a	2b	3	1	3	2b	1
Rumex acetosella	1	1	1		+	+	+	1	+	+	1	+
Remaining species												
Achillea millefolium								r				r
Amelanchier lamarckii			r		r							r
Anthoxanthum odoratum	+	+	+							+	+	+
Cerastium fontanum	r							+			+	
Crataegus monogyna											r	
Hieracium laevigatum						1			+			+
Hieracium umbellatum	+	+		r	+	+	r	+	r	r	+	+
Holcus lanatus											r	
Hypochaeris radicata	r	+	1	r	r	1	+	1	2m	r	1	2m
Jasione montana								r				
Leontodon autumnalis									+			
Luzula multiflora[1]				+	+	r	+	+				
Plantago lanceolata								+	r			
Prunus serotina		r	r			r			r		r	r
Quercus robur		+	r		r	r		r	r		r	r
Rhamnus frangula		+	r		+	r	r	+	r	r	+	
Sorbus aucuparia	r	r	r	r	+				r			
Taraxacum officinale s.s.								+		r	r	

Table 4 Continued

4C Grassland Experimental plot	C 1	C 1	C 1	C 2	C 2	C 2	L	L	L	D	D	D
Year	1990	1995	1995	1990	1995	1995	1990	1995	1995	1990	1995	1995
Sod cut	-	-	+	-	-	+	-	-	+	-	-	+
Vegetation cover (%)	80	95	85	90	95	85	92	90	90	92	80	90
Number of species	25	21	30	23	29	27	25	27	31	28	27	25
Matgrass sward species												
Agrostis canina	2m	2b	2b	2m	2b	2b	2m	2b	2a	2m	2b	2b
Agrostis capillaris	2m	2a	+	+	2a	1	2a	2a	2m	2m	2m	2a
Arnica montana *	1	+	2m	+	+	r			+			
Danthonia decumbens *	r	+	+	2a	2a	2a	2a	2a	+	2a	2a	
Galium saxatile *	2b	2b	2m	2m	2m	1	2m	2a	2m	2m	2a	1
Hieracium pilosella	+	2a	3	2b	2b	2m	+	1	2a	2m	2a	+
Nardus stricta *		r				r						
Polygala serpyllifolia	+		1	+	r	+	+	r	1			1
Potentilla erecta *	1	2a	+	+	+	+	2m	2a	+	1	+	+
Succisa pratensis					r	r						
Veronica officinalis *				+	2m			r		+	2m	
Heathland species												
Calluna vulgaris #	r	+	2a	2a	2a	3	2a	2a	3	2a	2a	2a
Empetrum nigrum							+	+		r	+	
Erica tetralix			r		+		r	+	r	r	r	
Genista anglica #			+		+				+	r	+	
Vaccinium vitis-idaea				+	+							
Frequently found species in matgrass swards and heathland vegetations												
Carex pilulifera	2m	+	2m	2m	2m	2m	2m	2m	2a	2m	2m	2m
Deschampsia flexuosa	+	1	+		+	+	2a	2a	+	r	+	+
Festuca ovina	3	3	2a	3	2b	2a	3	2b	2b	3	2b	2a
Molinia caerulea	+	2a			+	+	+	+	+			+
Rumex acetosella	1	+	1	1	+	2m	1		1	1	+	1
Remaining species												
Achillea millefolium			r	+		r		+	+	2m	2m	+
Aira praecox			2m			2m			2m			2m
Anthoxanthum odoratum			+	2m	1	+	+	1	+	2m	2m	+
Cerastium fontanum									r			
Chamerion angustifolium	+		+	r	+	+	+	r	+	1	+	+
Gnaphalium sylvaticum			+						+			
Hieracium laevigatum	1	2m	+	+	1	+	2a	2a	1	1	2m	+

4C Grassland												
Experimental plot	**C 1**	**C 1**	**C 1**	**C 2**	**C 2**	**C 2**	**L**	**L**	**L**	**D**	**D**	**D**
Year	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**	**1990**	**1995**	**1995**
Sod cut	-	-	+	-	-	+	-	-	+	-	-	+
Hieracium umbellatum	r	+	+	+	+	r	+	+	+	+	+	r
Hieracium vulgatum					+					r		
Holcus lanatus	2m	+	1		+	+	+	+	+	+	+	+
Hypochaeris radicata	2a	2a	2m	2a	2m	3	2b	2a	2b	2b	2a	3
Jasione montana			r						+			
Leontodon autumnalis			+	r					+	r	+	
Luzula multiflora[1]	+	1	+	1	1	+		+				
Plantago lanceolata									+	r	+	+
Poa pratensis						r						+
Prunus serotina	+	+	+	r	r	r	r	+	r	r	+	r
Quercus robur	+											
Rhamnus frangula	r		+		r		r	+	r	r	r	
Rubus fruticosus	2a	3	r				+	+		r	r	+
Sorbus aucuparia							r	r				
Stellaria graminea	r											

end of the experimental period. In contrast, the other dominant dwarf shrub, *Empetrum nigrum*, failed to significantly reestablish after sod removal in the Heath, and did not even return in one sod cut, unlimed plot. The characteristic heathland species *Genista anglica* increased in abundance after sod cutting of the Matgrass sward.

The abundances of *Molinea caerulea* were lower in 1995 in most sod cut plots than in the intact vegetation in 1990 and 1995. In contrast, *Agrostis canina* was more abundant in the sod cut, unlimed plots in 1995, regardless of the original vegetation.

LIMING

Similarly to the soil chemical parameters, no major differences between liming with limestone or dolomite were observed. Therefore, dolomite and limestone treatments will be discussed together, unless stated otherwise.

Liming without sod cutting caused an increase in species number in both the Heath and the Matgrass sward, but not in the Grassland. In all experimental plots, most of the newly established species were characteristic for matgrass swards. In the Matgrass sward, new establishments of *Polygala serpyllifolia, Potentilla erecta, Nardus stricta* and *Hieracium pilosella* were found after liming. The expansion of *Agrostis canina* in the limed subplots of the Matgrass sward and Grassland was equal to the increase in the unlimed subplots of these sites. An increase in abundance of *Agrostis canina* was also observed in the limed Heath subplots, but it was absent in the unlimed subplots of this site.

The effects of the combination of sod cutting and liming were most pronounced in the Matgrass sward. The Heath vegetation responded to a lesser degree to this combination of treatments, whereas in the Grassland, the effects of a combined liming and sod cutting treatment were almost similar to the effects of sod cutting (Table 4, sod cut, limed subplots). In the Heath and Matgrass sward, the combination of both treatments increased species number considerably compared to the intact vegetation in 1990 and the unlimed sod cut subplots. Compared to sod cutting only, the number of characteristic matgrass sward species increased. Some of those species, e.g. *Potentilla erecta* and *Hieracium pilosella* had higher abundances than in either one of the treatments alone. Remarkable is the large abundance of *Hypochaeris radicata* in all sod cut, limed vegetation types. As in the unlimed sod cut plots, the dwarf shrubs *Calluna vulgaris* and *Genista anglica* benefited clearly from sod cutting, but no additional effect due to liming treatments was observed.

EFFECTS OF LIMING ON *ARNICA MONTANA* PERFORMANCE IN ESTABLISHED VEGETATION

Detailed observations on the *Arnica montana* population in the non-sod cut parts of the Matgrass sward, showed that the population in the unlimed subplots declined from 1991 until 1996 (Table 5). During this period, the number of rosettes in the unlimed subplots gradually decreased by almost 70%, as well as a decrease in the number of flower buds was noted. As an indication for the seed producing potential of the population, the ratio between flower buds and rosettes was calculated (‘flower/rosette ratio’). Although this flower/rosette ratio fluctuated between years, it was always less than 0.5. In the limed subplots, the *Arnica montana* population expanded during the same period, as can be seen from the rosette numbers (Table 5). In the same period, the number of flower buds increased even more, resulting in a higher flower/rosette-ratio (always > 0.5) in the limed compared to the unlimed vegetation. Limestone increased the number of rosettes more than dolomite, whereas the latter treatment improved the flowering density of *Arnica montana* more than in the limestone treatment.

In the Grassland, *Arnica montana* was only present in the control subplots. In these subplots, the number of rosettes increased from 1991 to 1995 by 62 % (from 25 to 41 rosettes per subplot, mean values). Since the flower bud number increased only by 7% during the same period, the flower/rosette ratio was somewhat lowered (from 1.93 to 0.58). Nevertheless, this ratio was similar to the limed subplots of the Matgrass sward and much higher than in the unlimed subplots of that site (Table 5).

EFFECTS OF LIMING ON GERMINATION AND SURVIVAL OF *ARNICA MONTANA*

Most *Arnica montana* seeds germinated during the first autumn after sowing in the sod cut subplots and only a few next spring; after this, no germination of *Arnica*

Table 5 Performance of *Arnica montana* in non sod-cut parts of the Matgrass sward during the period 1991-1996. Number of rosettes, flower buds and flower/rosette ratio. Rosettes and flower buds expressed as percentages from 1991, with absolute values in 1991 between brackets. Flower/rosette-ratios are derived from absolute values. C=control (no lime), L=limestone, D=dolomite.

	rosette			flower buds			flower/rosette		
	C	L	D	C	L	D	C	L	D
1991	100	100	100	100	100	100	0.21	0.51	0.47
	(99)	(72)	(82)	(20)	(37)	(39)			
1994	77	107	95	106	249	303	0.34	1.19	1.51
1995	51	153	111	31	186	197	0.12	0.63	0.85
1996	32	132	101	71	203	187	0.46	0.79	0.88

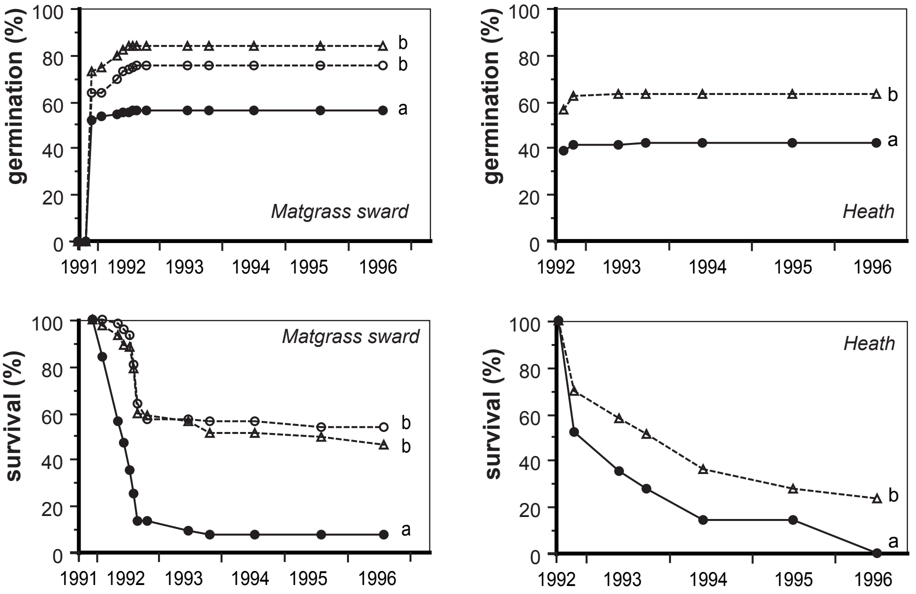


Figure 4 The effects of liming on germination and survival percentages of sown *Arnica montana* in the Matgrass sward and Heath. Mean percentages, N=4 (except in controls of Matgrass sward, N=8). Different letters indicate significant differences ($p<0.05$) between treatments within a plot. Closed circles: control (unlimed) subplots; open circles: limestone treated subplots; open triangles: dolomite treated subplots.

Table 6 Mean estimated biomass of sown, living *Arnica montana*-plants in June 1994, 1995 and 1996 in the sod-cut Matgrass sward and in the sod-cut Heath. Biomass is estimated by the multiplication of maximum leaf length, maximum leaf width and the number of leaves (mm^2). Significant differences in biomass between treatments per year (row) are indicated by different letters. *: in 1996, all plants in the control treatment in the Heath-site had died.

			Treatment			
	control		limestone		dolomite	
Matgrass sward						
1994	1370	ab	2568	b	958	a
1995	1844	a	4702	b	1394	a
1996	3261	a	7383	b	2437	a
Heath						
1995	533	b	.		299	a
1996	0*		.		580	

montana was observed (Figure 4). Between the limed and unlimed subplots, a clear difference in germination was observed: whereas 76% - 84% of the seeds germinated in the limed subplots, only 56% of the seeds germinated in the unlimed subplots of the Matgrass sward. In the sod cut Heath, germination of *Arnica montana* was somewhat lower after liming (63%) but still higher than in the control treatment (42%). A drastic effect of liming was also observed on the survival of the plants: four years after germination, only 8% of the *Arnica montana* plants were alive in the unlimed treatment, whereas 53% and 46% of the plants were still present in the limestone and dolomite treatments, respectively (Figure 4). Biomass production of the *Arnica montana* plants was clearly higher in the limestone treated subplots than in the unlimed and dolomite treated (Table 6). In the Heath, *Arnica montana* plants were even bigger in 1995 in the unlimed subplot than when dolomite was applied. All the plants in the unlimed subplot died during the next year, in contrast to the plants in the dolomite treatment. The first flowering of the sown *Arnica montana* plants in the Matgrass sward was noted in 1996, five years after the start of the experiment. No correlation between the percentage of flowering individuals with liming treatment was observed, but the statistics may have been biased by the very low number of plants in the control treatment. In the Heath, no flowering of *Arnica montana* was observed.

DISCUSSION

In this study we have tested restoration strategies used to rehabilitate the characteristic soil conditions and the typical species-rich vegetation of dry heathlands. The applied restoration strategies are based upon the ecological knowledge on characteristic heathland plant species. The main aim of the experiment was to develop adequate restoration practices for three threatened ecosystems: (1) eutrophied, dry species-poor heath, (2) acidified and eutrophied matgrass swards and (3) abandoned grasslands. We aimed to regenerate a CALLUNA-GENISTION PILOSAE community in the eutrophied Heath, and NARDO-GALION SAXATILIS communites in the other two sites.

Restoration of soil conditions and vegetation

It was proved that suitable soil conditions could be restored, either by sod cutting or liming or by a combination of both measures. Sod cutting is an appropriate method in restoring the nutrient-poor soil conditions required by dry heath vegetation (this study, Diemont & Linthorst-Homan 1989). Ecosystem functioning is generally affected by sod cutting in two ways: firstly, nutrient concentrations in the soil are reduced to sufficiently low levels for heath vegetations (Table 1; Houdijk *et al.* 1993). Secondly, a bare substrate for germination and seedling establishment is created by sod cutting.

Sod cutting reduced almost all nutrient concentrations in the top soil layer: not only were N and P reduced, but Ca and Mg concentrations were also lower in the sod cut than in the non-sod cut parts. Although these base cations are important to the buffering capacity of these acid soils (Scheffer & Schachtschabel 1992), pH was not influenced by sod cutting in the Matgrass sward and Grassland. In the Heath, pH of the mineral top 10 cm soil increased due to the removal of the most acidic organic top centimetres. The initial decrease in mineral nitrogen immediately after the sods have been removed was, however, followed by a large increase in mineral nitrogen to up to three times the nitrogen concentration in the non-sod cut soils (Figure 1). This might be due to an initial increase in nitrogen mineralisation; Berendse (1990) measured high N mineralisation rates during the first two years after sod cutting, which were probably caused by the mineralisation of roots that were excised with the removal of the sods. Since these remaining living roots have relatively high N contents, the C:N-ratio of the organic material in the soil is temporarily decreased, which consequently leads to an increase in mineralisation (Berendse 1990). As plant uptake of N is negligible during the first year after sod cutting, the mineral nitrogen formed by the mineralisation can reach high values in the top soil. High ammonium concentrations may arise from the fact that in acidic heathlands, nitrate is almost exclusively formed in the humus layer, whereas the formation of ammonium also occurs in the mineral soil layers (De Boer *et al.* 1989). Removal of the organic soil layers thus negates the nitrification capacity, resulting in enhanced ammonium concentrations. These high ammonium concentrations may affect the establishment of *Arnica montana* in the first year after sod cutting, since these are detrimental to this species (De Graaf *et al.* 1998). The establishment of more acid tolerant species, e.g. *Calluna vulgaris*, *Empetrum nigrum*, *Deschampsia flexuosa* and *Molinia caerulea*, will probably be unaffected due to their preference for ammonium as a nitrogen source (Troelstra *et al.* 1995, De Graaf *et al.* 1998). After the first year following sod cutting, the mineral nitrogen concentrations decreased to values below those in non-sod cut plots (Figure 1).

In dry heath vegetations, gap formation is important for the establishment of seedlings of many species (Miles 1974, Fennema 1990). Experiments on sod cutting in Scottish heath showed that most species present in the original vegetation had returned three years after sod removal (Miles 1973). In addition, he observed that a higher number of species establishment occurred on more fertile, less acidic heathland soils than on the more acid soils, including some typical matgrass sward species (e.g. *Galium saxatile*, *Veronica officinalis*, *Polygala serpyllifolia* and *Potentilla erecta*). Our results are in agreement with this: after sod cutting nearly all species returned and most species established on the Grassland, followed by the Matgrass sward, whereas the least number of species established on the Heath. Most of these species were present in the plot before sod cutting and are thus likely to have emerged from the soil seed bank (Table 4). Other newly found species were either present in the surroundings of the plot or were ruderals (Grassland).

Apart from the species number, the relative abundance of species is also affected by sod cutting. As in many species-poor heathlands, the abundance of *Deschampsia flexuosa* and/or *Molinia caerulea* is largely reduced in favour of *Calluna vulgaris* (Table 4; Diemont & Linthorst-Homan 1989). The establishment of *Empetrum nigrum* was, however, seriously restricted by sod cutting. This indicates that in grass heaths formerly dominated by *Empetrum nigrum*, sod cutting should be applied only after carefull consideration.

In accordance with our hypothesis, the decline of the *Arnica montana* population in the non-sod cut Matgrass sward continued throuhout the experimental period (Table 5). This decline was probably due to soil acidification: during the experimental period, base cation concentrations in the unlimed, non-sod cut subplots were reduced by 50% (Figure 3) and ammonium concentrations increased to values markedly above those on which well developed NARDO-GALION SAXATILIS communities usually occur (De Graaf *et al.* 1994, Roelofs *et al.* 1996). Moreover, it is unlikely that the decline *Arnica montana* is caused by competitive interactions with grasses, since the total cover of the grasses in the controls of the non-sod cut plots did not change considerably during the experimental period.

Liming served to reverse soil acidification, especially in the Heath plots and in the sod cut parts of the Matgrass swards. Liming had little effect on top soil chemistry of the formerly limed, probably due to the low dose applied. In general, soils of Heath and Matgrass sward became less acidic as is indicated by the higher pH and base cation concentrations. Soil conditions in the Matgrass sward now closely resemble those on which vital NARDO-GALION SAXATILIS communities occur (Houdijk *et al.* 1993, Roelofs *et al*. 1996). The decrease in the Al/Ca-ratio to values below 3 is beneficial to *Arnica montana* since high aluminium concentrations and high Al/Ca-ratios are known to have a detrimental effect on *Arnica montana* (Heijne 1995, De Graaf *et al.*

1997). The beneficial effects of liming on *Arnica montana* are clearly shown by the improved vitality of the established population in the Matgrass sward (Table 4) and the better germination, establishment and vitality of *Arnica montana* sown in the sod cut parts of the Heath and Matgrass sward (Figure 4, Table 6). Moreover, liming increased the species number, which was largely due to an increase in species characteristic of NARDO-GALION SAXATILIS communities, again indicating that these species favour less acidic soils than the characteristic CALLUNA-GENISTION PILOSAE species. The tolerance of some of the latter species, e.g. *Calluna vulgaris* and *Deschampsia flexuosa,* to high aluminum and ammonium concentrations which occur on very acid soils have been shown in several studies (Hackett 1965, Troelstra *et al.* 1996, De Graaf *et al.* 1997).

The effects of lime applications on ecosystem functioning are, however, not always beneficial. Firstly, not all species respond well to liming: the abundance of *Calluna vulgaris* is largely reduced in the limed plots of the Matgrass sward. A reduction in abundance of acid-tolerant species after liming has previously been observed by Blom & Wincent (1990) in acidic grasslands in Sweden. Secondly, liming may enhance N-mineralisation and nitrification (Nyborg & Hoyt 1978), which can eventually lead to leaching of nitrate to the groundwater (Kreutzer 1995) and to a transition from dwarf-shrub dominated heath to monotonous grass stands (Heil & Aerts 1993). Although we did not measure mineralisation rates in this experiment, some observations indicate that nitrogen mineralisation increased, at least temporarily, in the limed plots. The most obvious indications for the enhanced nitrogen mineralisation and nitrification were the increased nitrate concentrations in the winters of 1994 and 1995. It is likely that the vegetation readily took up this nitrate during the following summer, since the nitrate concentrations in the limed plots did not differ from those in the unlimed plots anymore in autumn. Leaching of NO_3^- only occurs if the retention capacity of the ecosystem for nitrogen is exceded (Kreutzer 1995). Nitrate leaching from heathlands is very low (Van der Maas 1990). It is probably even lower in sod cut heaths, since it was found that after sod cutting of a species-poor dry heath, all incoming atmospheric nitrogen accumulated in the system for a period of 30 years (Berendse 1990). Nevertheless, we recommend a combined liming and sod cutting treatment in order to minimise the risk of unwanted eutrophication effects.

Chemical conditions in the top soil of the Grassland plots meet the requirements for a NARDO-GALION SAXATILIS vegetation even without liming or sod cutting (Fennema 1992, Houdijk *et al.* 1993, Roelofs *et al.* 1996). Species numbers were highest in these plots, both in general and for the characteristic NARDO-GALION SAXATILIS species. Moreover, the *Arnica montana* population in the unlimed, non-sod cut plots is as healthy as in the limed plots of the Matgrass sward and the species is rapidly expanding in the unlimed sod cut plots (Table 4). It is probable that the past lime applications still affect ecosystem functioning in the abandoned Grassland. Effects of liming on soil pH, exchangeable calcium and possibly on mineralisation, are detectable long after liming has been ceased and may hamper heathland restoration on abandoned fields

(Pywell *et al*. 1994). In this study, experimental liming of the Grassland plots with low doses had little additional effect on soil chemistry, nor did it influence the species composition. Soil conditions in this abandoned grassland are now becoming suitable for plant species adapted to nutrient poor, slightly acidic soil conditions. Due to the practice of yearly mowing of the vegetation with removal of the hay, soil fertility has slowly decreased since the cessation of agricultural practices. Despite this, many species which are characteristic of more fertile grasslands are still present, although some of these species, e.g. *Holcus lanatus*, are now declining.

Sod cutting has accelerated the restoration of heathland vegetation on abandoned grassland (Table 4): especially the abundance of *Calluna vulgaris* was increased by sod cutting. One may, however, question how necessary an expensive restoration method such as sod cutting is for the restoration of abandoned grassland, when considering that the less costly method of mowing with the removal of hay is also capable of restoring a NARDO-GALION SAXATILIS vegetation.

Concluding remarks

It is concluded that restoration of the soil conditions is necessary for the rehabilitation of various heath vegetations. The combination of sod cutting and liming is the most successful method for the rehabilitation of acidified NARDO-GALION SAXATILIS communities, because it offers optimal conditions for germination and provides appropriate soil conditions for the growth of the characteristic species. Moreover, the probability of enhanced nitrogen mineralisation is likely to be negligible, thus reducing the risk of enhanced growth of grasses. Sod cutting reduces the availability of soil nutrients and creates optimal germination and establishment conditions for many heathland species. Furthermore, it reduces the relatively large abundance of the grasses in the heath.

Transformation of species-poor heaths to a species-richer heathland vegetation can only be partly achieved by a combination of sod cutting and liming. We were unable to convert the CALLUNA-GENISTION PILOSAE vegetation to one in which many NARDO-GALION SAXATILIS species are present. It is, of course, obvious that if the long-term conservation of the restored heathland vegetation is to be assured, the inputs of atmospheric pollutants must decrease below set critical loads (e.g. Bobbink & Roelofs 1995). Abandoned grasslands which still possess a sufficient buffering capacity, primarily need a reduction of soil nutrients to develop species-rich stands. This can be achieved through mowing or, more rapidly, through sod cutting.

The success of restoring species-rich heathland vegetation is, however, not only dependent on the restoration of abiotic conditions. Reestablishment of endangered species also depends on the availability of seeds. A positive situation exists in the location of this, but seed availability is a limiting factor in many restoration projects. In this experiment we did not observe the establishment of NARDO-GALION SAXATILIS species that were absent from the seed bank or in the environs of the experimental plots. Even *Thymus serpyllum*, which was found 300-500 m from the experimental plots, was not found after rehabilitation of the soil. As soil conditions were suitable for the establishment of this and other species characteristic of this community (Houdijk *et al.* 1993, Houdijk 1993), the absense of the species must be caused by limited dispersal and/or by absence of seeds in the seed bank. Unfortunately, most of the rare species of the NARDO-GALION SAXATILIS comunities lack long-term seed banks (Bakker *et al.* 1996), thus diminishing the chances for successful reestablishment after restoration of the soil. This is sadly shown by the results of a similar experiment with combined sod cutting and liming in another Dutch heathland: although we were able to restore soil conditions, the rare NARDO-GALION SAXATILIS species, which had disappeared ten years before, did not return due to the absence of viable seeds or nearby seed sources (Roelofs *et al.* 1996). This emphasises the importance of saving some seed setting plants from restoration practices such as sod cutting, in order to provide nearby seed sources after restoration. Otherwise, deliberate reintroduction of plant species is probably the only way to establish complete NARDO-GALION SAXATILIS communities in heathlands in which soil conditions have been restored in the near future .

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CHAPTER 7

SYNTHESIS

SYNTHESIS

THE CALCICOLE - CALCIFUGE GRADIENT

The distribution of heathland species and vegetations over soils of different pH values can be considered as a distribution along a calcicole - calcifuge gradient. Such gradients are observed in many ecosystems, including wet and dry heathlands (Balme 1956, Hayati & Proctor 1990, De Graaf *et al.* 1994, Roelofs *et al.* 1996, chapter 2).

Calcicole behaviour of plants can be described as the ability of plants to establish, grow and compete successfully on calcareous soils (Tyler & Ström 1995), whereas a comparable description can be given for the calcifuge behaviour of plants on acid soils. It is generally held that both the calcareous and the acid environment require special, physiological adaptations. Calcareous soils generally have a very low availability of phosphorus and iron and it is thought that, as the calcifuge species are unable to solubilize adequate amounts of Fe from these soils, they may become iron deficient (Kinzel 1982). Another explanation for the iron deficiencies observed in calcifuge plant species, is their inability to retain iron in a metabolically active form in the tissues (Kinzel 1982). It has also been reported that phosphorus would be the primary limiting factor for growth for the calcifuges *Rumex acetosella* and *Silene rupestris* in a calcareous environment (Tyler 1992), whereas calcicole species are often highly efficient in phosphorus uptake (Musick 1978). Furthermore, Balme (1956) reported that experiments with the calcifuge *Viola lutea* showed an inability to take up adequate amounts of potassium. Hence, the lack of adaptations for the uptake of essential nutrients, resulting in deficits of these nutrients, is likely to exclude the calcifuge species from calcareous soils.

Calcicole behaviour is thought to be associated with the intolerance of plants to high concentrations of H^+, Al^{3+}, Mn or Fe, conditions prevailing in acid soils (Runge & Rode 1991, Marschner 1991). Intolerance to high ammonium concentrations could be added to this list, as ammonium is the predominant mineral nitrogen form in acid soils and a toxicity syndrome for ammonium has been described (Gigon & Rorison 1972, Mehrer & Mohr 1989). The work described in this thesis provides further insight into the distribution of species along the calcicole - calcifuge gradient, especially about factors determining the species distribution at the acid extreme of the gradient in heathlands.

The heathland species of acid habitats, such as *Calluna vulgaris*, *Erica tetralix*, *Deschampsia flexuosa*, *Empetrum nigrum*, can without doubt be classified as calcifuge species. They are clearly adapted to the prevailing conditions of their habitats, as is shown by the preference for ammonium over nitrate as an N-source (Houdijk 1993, Troelstra *et al.* 1995, chapter 3), and the tolerance to high aluminium and high ammonium concentrations (Hackett 1965, chapters 3, 4). As such, the characteristic species of the less acidic, species-rich heathlands can be considered as calcicole species, as they lack the adaptations of the calcifuges. The species of species-rich heathlands generally prefer a mixed nitrogen nutrition, in which ammonium concentrations are not too high (Gigon & Rorison 1972, Falkengren-Grerup & Lakkenborg-Kristensen 1994, Dijk *et al.* 1997, chapters 3, 5). Furthermore, they are sensitive to high aluminium concentrations as well as to high aluminium-calcium ratios (chapters 4, 5). It was demonstrated that the poor development of *Cirsium dissectum* and *Arnica montana* on high aluminium and/or ammonium concentrations correlated with important reductions in the uptake of major nutrients, particularly of calcium, magnesium and potassium (chapters 3,4,5). A comparison between the nutrient contents of plants grown under natural conditions in the field and the plants treated with ammonium and aluminium, showed that the latter had much lower contents of calcium, magnesium and potassium, indicating deficiencies of these nutrients (chapters 3, 5). Hence, it is concluded that these two species of the species-rich heathlands are not adapted to the environmental conditions prevailing in very acid soils.

Yet, one may question whether the plant species of the species-rich heaths can be regarded as true calcicoles. In other words, can species that do not show calcifuge behaviour automatically be considered as calcicoles? Or should we consider the existence of a third, intermediate group, as has been suggested by Balme (1956)?

There are some indications for the existence of such an intermediate group, which come from field observations as well as from physiological experiments. The different heath vegetations, especially the wet heath vegetations, are often part of a larger gradient, ranging from the dwarf-shrub dominated heath vegetations to fen meadows or vegetations of even more calcareous soils (Balme 1956, Boeye & Verheyen 1992, De Graaf *et al.* 1994, Jansen *et al.* 1996). In fact, the wet heath vegetations that are described in chapter 2 are part of such a gradient, extending from the dry, *Calluna vulgaris* dominated species-poor heath on the most acid parts, via the species-poor and species-rich wet heath in Caricion nigrae communities (De Graaf *et al.* 1994). The latter vegetation type developed on the least acid soils. It is likely that abiotic factors related to soil pH and/or hydrology cause the difference in species distribution between the species-rich heathlands and the vegetation of more calcareous soils. Phosphorus availability could be one of these factors.

The distribution of the species-rich heath vegetation types and their characteristic species is almost restricted to soils buffered by cation exchange processes (Hayati & Proctor 1990, Houdijk *et al.* 1993, De Graaf *et al.* 1994). In such soils, phosphorus availability is often much better than in more acid or more calcareous soils. In the more acid soils, phosphorus precipitates with aluminium to $AlPO_3$-complexes, whereas in more calcareous aerobic soils, the availability of phosphorus to plants is limited by the complexation of phosphorus with calcium or iron. Thus, it is unlikely that the species-rich heathland species need adaptations for the uptake of phosphorus, as do the true calcicoles soils (Tyler & Ström 1995).

Houdijk (1993) performed some experiments with *Thymus serpyllifolia*, a characteristic species of dry, species-rich heathlands. It was shown that the plants could survive at low pH (4 < pH < 5), as long as ammonium and nitrate nutrition were balanced (Houdijk 1993). Imbalanced high ammonium concentrations were detrimental to *Thymus serpyllifolia,* indicating that the species showed calcicole behaviour similar to that of *Arnica montana* and *Cirsium dissectum*. However, the vitality of *Thymus serpyllifolia* was also severely reduced when the pH of the soil increased to values above 7. Hence, *Thymus serpyllifolia* can also not be regarded as a true calcicole, and the existence of an intermediate group of species regarding calcifuge or calcicole behaviour is strongly suggested by the results of this experiment.

Balme (1956) suggested that the characteristic species of the intermediate habitats of calcicole - calcifuge gradients simply lack adaptations to either the acid or calcareous soil conditions. This is in agreement with the results described in this thesis for the acid end of the gradient, as aluminium and ammonium have been shown to be toxic. Yet, despite the overall similarity in sensitivity to aluminium and ammonium, differences in the response of *Arnica montana* and *Cirsium dissectum* were also observed. In general, *Arnica montana* was more tolerant to ammonium than *Cirsium dissectum*, whereas the latter species could resist higher aluminium concentrations. Hayati & Proctor (1990) studied the uptake of nutrients in a number of wet heath species occurring in the calcicole - calcifuge gradient. They observed that in this wet heathland gradient 'every species and every element behaved differently', with regard to nutrient uptake. They concluded that the differing patterns in nutrient uptake reflected differentiation in niches between the species. Analogously, the difference in the response of *Arnica montana* and *Cirsium dissectum* to aluminium and nitrogen also suggests a degree of habitat specialisation, which might for instance be related to the prevailing soil moisture content of their habitats. Thus, although the species of the intermediate group of the calcicole - calcifuge gradient may not be adapted to acid or calcareous soils, they seem to be highly specialised to the environmental conditions of their habitats.

CAUSES FOR THE RECENT DECLINE OF THE HEATHLAND VEGETATIONS

The majority of the typical species of the species-rich heathlands have been declining during the last decades. This recent decline has been related to the effects of atmospheric deposition or, as far as wet heathlands are concerned, to changes in hydrology (Van Dam *et al*. 1986, Dueck et al. 1992, Dueck & Elderson 1992, Fennema 1992, Aerts & Heil 1993, Heijne 1995, Jansen *et al*. 1996, Roelofs *et al*. 1996, this thesis). Atmospheric deposition of sulphur causes soil acidification, but deposition of nitrogen may have several effects. Three main effects of atmospheric deposition of N can be distinguished in heathlands (Bobbink *et al.* 1998): i) a quantitative aspect, e.g. eutrophication due to the increased accumulation and availability of nitrogen, ii) soil-mediated effects of acidification, and iii) increased sensitivity to secondary stress factors.

When changes in hydrology result in a decreased input of alkalinity and base cations by groundwater into the upper soil layers, acidification of these layers may occur. Furthermore, as the top soil desiccates, mineralisation of nitrogen is accelerated, resulting in increased availability of mineral nitrogen (Grootjans *et al.* 1986). In addition, desiccation of the upper soil layers itself may cause plants to wilt or die.

The deterioration of the species-poor heathlands in the Netherlands has been caused by the accumulation of nitrogen due to atmospheric deposition (Roelofs 1986, Berendse 1990, Van Vuuren 1992, Aerts & Heil 1993, Bobbink *et al.* 1998, Lee & Caporn 1998). Furthermore, it has been reported that the *Calluna vulgaris*-dominated heathlands have become more sensitive to secondary stress factors, such as frost injury and plagues of heather beetles (*Lochmaea suturalis;* Berdowski 1993, Bobbink *et al.* 1998, Power *et al.* 1998, Carroll *et al.* 1999). The opening of the canopy following heather beetle plagues has been shown to be essential in the transition from *Calluna*-dominated heathlands to heathlands dominated by grasses (Berdowski 1993).

Furthermore, deposition of $(NH_4)_2SO_4$ causes soil acidification (Van Breemen *et al.* 1982). However, as the characteristic species of the species-poor heaths are adapted to acid conditions, it is unlikely that they would suffer from the soil acidification.

Dry species-rich heathlands

This is not the case for the species-rich heaths. Both physiological (chapters 3, 4, 5) and correlative field studies (Van Dam *et al.* 1986, Fennema 1992) strongly suggest that soil acidification is a major threat to these vegetations. One may question, however, whether soil acidification is actually occurring in species-rich heathlands and thereby whether the decline of the species-rich heaths can be attributed to soil acidification.

Actual input of protons in these ecosystems is either directly through atmospheric deposition, or indirectly through nitrification or through the uptake of ammonium. Nitrification is known to occur in species-rich heathlands and related acidic grasslands, but is very low in heathland ecosystems (Van Vuuren *et al.* 1992, Morecroft *et al.* 1994, chapter 2).

The uptake of large quantities of ammonium leads to acidification of the rhizosphere, as ammonium uptake is associated with the excretion of H^+ (Raven & Smith 1976, chapter 5). Both *Arnica montana* and *Cirsium dissectum* preferentially take up ammonium from a mixed nitrogen source (chapter 5). A concomittant acidification of the nutrient solution has been observed for *Arnica montana* (chapter 5), but not for *Cirsium dissectum*. We hypothesised that the lack of H^+-excretion by this species was due to the high external H^+-concentration and it was thought that *Cirsium* could not excrete H^+-ions against such a steep gradient. However, when the external pH is higher, *Cirsium dissectum* might be able to excrete protons in order to counterbalance the uptake of ammonium. Experiments by Pierik (1997) support this hy-

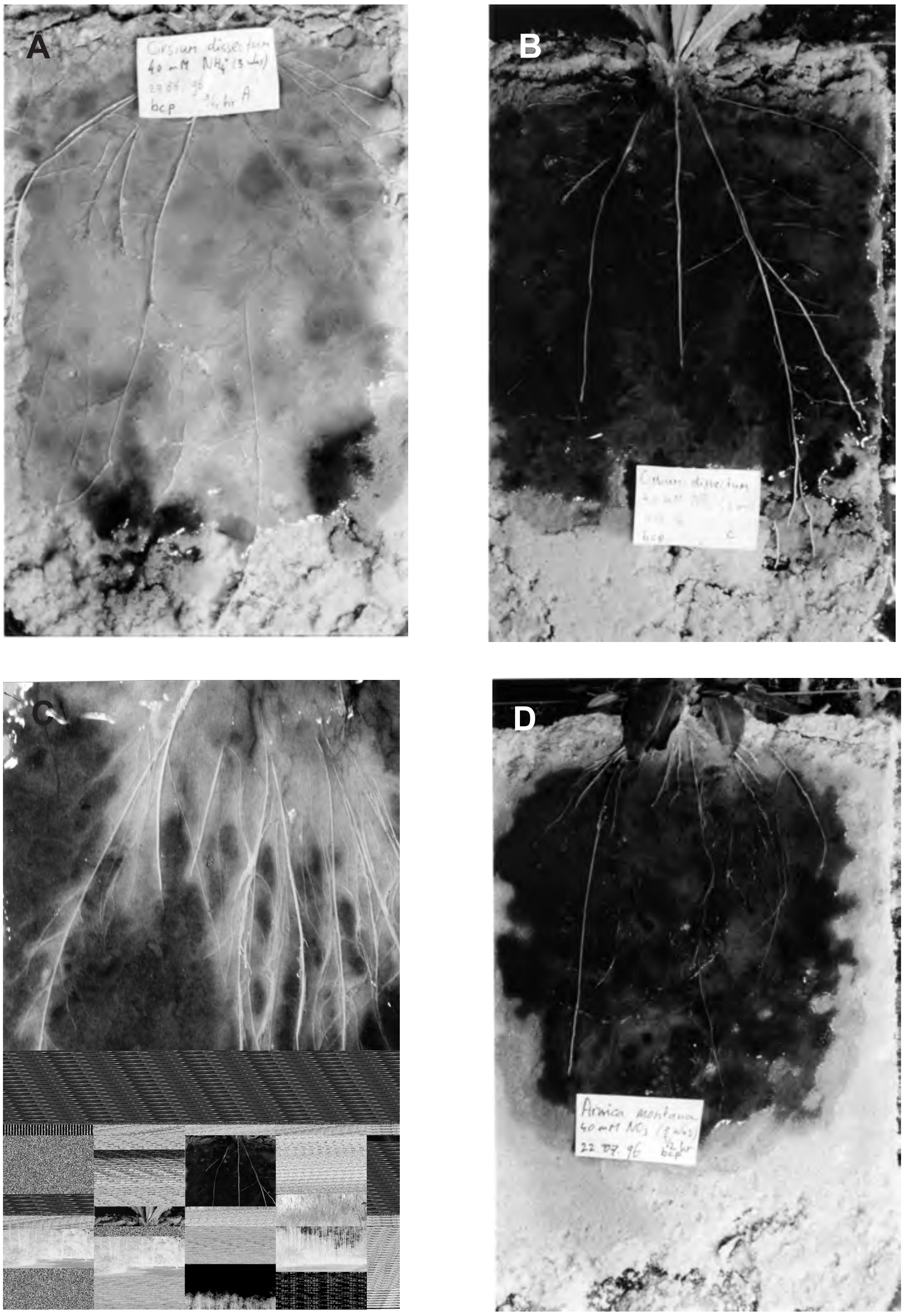

Figure 1 Soil pH in the rhizosphere of *Cirsium dissectum* (A, B) and *Arnica montana* (C, D) grown at nitrate (B, D) or ammonium (A, C) nutrition. Light rhizospheres: pH<5.2, dark rhizospere: pH>6.8. (Pierik 1997)

pothesis. He observed a decrease in soil pH from values higher than 6.8 to pH values below 5.2 in both *Arnica montana* and *Cirsium dissectum*, when they were supplied with NH_4^+-nutrition (Figure 1). In contrast, when supplied with nitrate as a sole N-source, the pH of the soil solution remained high.

The acidification of the rhizosphere by ammonium uptake may, however, be limited, when both ammonium and nitrate are available to plants. In soils, the uptake of ammonium will lead to depletion of ammonium in the rhizosphere (Marschner *et al.* 1991). With the depletion of ammonium, plants will probably switch to nitrate uptake, as nitrate becomes more available to plants (Boxman *et al.* 1991). However, when nitrification is low, nitrate availability will be low and plants will continue to take up ammonium and thus increasingly acidify the rhizosphere.

The acidification of the rhizosphere as a result of ammonium uptake may cause an increase in aluminium concentrations of the rhizosphere on the one hand, while on the other hand, major nutrients such as calcium, potassium and magnesium may leach out of the rhizosphere. Leaching of calcium, magnesium and potassium might be particularly harmful to the plants, as it hampers the uptake of these essential nutrients and thus adds to the negative effects of ammonium nutrition and aluminium toxicity (chapters 3,4,5).

Wet species-rich heathlands

There are less studies on the decline of the species-rich wet heathlands in relation to soil acidification than on the decline of the species-rich dry heathlands. To my knowledge, no field data are available on the actual decline of characteristic wet-heath species in relation to soil acification. Yet, Lamers *et al.* (1998) performed a laboratory experiment on the effects of acidification on a minerotrophic wetland ecosystem, which relates to the species-rich wet heathlands. In intact soil cores, consisting of the upper 10 cm of the soil and vegetation, it was shown that both aluminium and ammonium concentrations in the soil solution increased to values that were shown to be toxic to *Cirsium dissectum* (resp. 125 µmol l^{-1} Al and over 500 µmol l^{-1} NH_4^+). At the same time, the pH in the soil solution fell from 7 to 4.5, whereas nitrate was no longer detectable. These results showed that the conditions that are so detrimental to *Cirsium dissectum*, e.g. unilateral ammonium nutrition in combination with low pH, are likely to occur in a field situation.

In this experiment, soil acidification was due to the combined effects of groundwater pollution with sulphur and desiccation. Hence, the study shows the potential risk of lowering groundwater tables in areas where high sulphate concentrations occur in the soil solution. Pollution of ground and surface water with sulphate has been shown to occur in Dutch minerotrophic fens (Jansen & Roelofs 1996). As these minerotrophic fens are often found in the direct vicinity of species-rich wet heaths, pollution of the wet heathlands with sulphur via the groundwater might also occur.

Without sulphur pollution, desiccation of the upper soil layers during a short period of time does not lead to the harmful combination of low pH values and high ammonium concentrations (Lamers *et al.* 1998, chapter 2). However, a permanent lowering of the groundwater tables will eventually lead to soil acidification, as the supply of base cations and alkalinity by the groundwater will cease and acidifying processes as ammonium uptake and nitrification will continue.

It is concluded that soil acidification, either due to atmospheric input of N and S, or due to lowering of the groundwater table, is a serious threat to the species-rich heathlands and is likely to occur. Future, long-term monitoring of both vegetation and soil processes is, however, needed to demonstrate actual acidification of these ecosystems under natural conditions.

Other threats to species-rich heathlands

Acidification is probably not the only factor threatening species-rich heathlands. In species-rich heathlands that are sufficiently buffered against soil acidification, increased nitrogen availability may lead to a similar dominance of grasses as in the species-poor heathlands, although the grass species may differ (Bobbink *et al.* 1998). This hypothesis has been supported by competition experiments with *Arnica montana*, *Viola canina* and *Agrostis canina,* which showed that, under circumstances of increased N-availability, *Agrostis canina* may become a stronger competitor (Dueck & Elderson 1992). However, further studies are needed in order to show the relative importance of soil acidification and changes in competition in the decline of the species-rich heaths. It is most likely that both processes occur, and, depending on specific site characteristics such as base saturation and management, either soil acidification or vigorous growth of grasses will finally cause the heathlands to deteriorate.

In addition to the effects of soil acidification and changes in competiton, increased nitrogen availability may lead to increased sensitivity to secondary stress factors, such as heather beetle plagues, frost and drought (Bobbink 1998). Reduced tolerance to drought as a result of increased N-availability is generally thought to be an effect of reduced root growth (Marschner 1991). High nitrogen availability reduces the need of an extensive root system and plants allocate biomass to the shoot, resulting in increased shoot to root ratios. These plants are, however, more sensitive to drought, as their evaporation increases, whereas the uptake capacity for water becomes decreased.

Moreover, an experiment by Verbakel (1993) with *Arnica montana* and *Cirsium dissectum* on drought resistance, showed that differences in mineral nitrogen nutrition also led to differences in drought tolerance. In fact, plants grown with ammonium as a sole N-source wilted at a higher soil moisture content than plants grown on nitrate or mixed N-nutrition (Figure 2). *Arnica montana* plants that had been cultured with nitrate as the sole N-source could endure significantly longer periods of

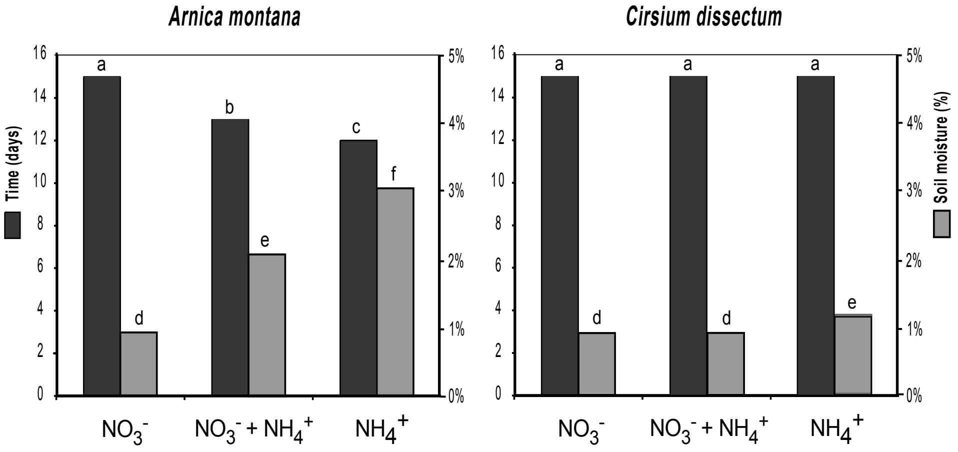

Figure 2 Effects of desiccation on *Arnica montana* and *Cirsium dissectum* which had been pretreated with NO3$^-$, NH4$^+$ or a mixture of NO3$^-$ and NH4$^+$. Desiccation effects are expressed as % soil moisture when wilting becomes obvious and time until wilting. Significant differences between treatments within species are expressed by different letters. (Verbakel 1993).

drought before wilting than plants grown on ammonium, while plants grown on both nitrogen forms endured intermediate periods of drought. As low concentrations of ammonium nutrition lead to increases in shoot-root ratios (chapter 3), the mechanism leading to the reduced drought tolerance is likely to be similar to that of increases in N availability.

RESTORATION OF HEATHLANDS

At present, many efforts are being made to conserve heathland ecosystems, as they are an important aspect of many West European landscapes. Moreover, especially the species-rich heathlands represent an important part of the plant diversity of low-nutrient ecosystems. The characteristic ecosystems of low-nutrient soils are, however, becoming increasingly rare, as human impact through intensified land use and changes in hydrology, ground and surface water pollution and atmospheric deposition of pollutants has become almost ubiquitous in western Europe. Attempts are now being made to rehabilitate deteriorated heathlands in addition to the conservation of the relicts.

It has been shown that good results in the rehabilitation of heathlands can be obtained whenever the processes that cause the heathlands to decline are counteracted. As the decline usually starts with changes in the top soil or hydrology, succesful restoration management acts to recreate the original abiotic conditions. Many restoration projects have shown that eutrophied heathlands can be successfully rehabilitated by removing the excess of nitrogen by sod-cutting (e.g. Helsper *et al*. 1983, Werger *et al*. 1985, Aerts & Heil 1993, Roelofs *et al*. 1993, chapter 6). Sod-cutting is especially successful in restoring the species-poor, dwarf shrub-dominated *Calluna* and *Erica* heathlands.

In acidified heathlands, counteracting soil acidification, e.g. by liming or reinforcing base-rich groundwater into the upper soil layers has proven again to be successful (Roelofs *et al.* 1993, Jansen *et al.* 1996, Roelofs *et al.* 1996, chapter 6). It has been shown by field experiments (De Graaf *et al.* 1994, Janssen *et al.* 1996, chapter 6), that, although increasing base saturation in the top soil of acidified species-rich heaths should in theory be satisfactory, combining measurements against acidification with sod-cutting clearly gives the best results. It has been shown that increasing the base saturation of the soil layers without sod-cutting, might lead to enhanced mineralisation, thereby not restoring the original nutrient-poor conditions (Pywell *et al*. 1994, chapter 6). Furthermore, liming without sod-cutting did improve the vitality of the existing population of endangered species in the Schaopedobbe experiment (chapter 6), but did not lead to an increased number of seedlings of e.g. *Arnica montana*. A number of experiments on germination of heathland species shows that the re-establishment of both rare and common species is inhibited by dense vegetations (Miles 1973, Fennema 1990, Bruggink 1993, chapter 6). It is thought that sod-cutting of heaths is not only important in removing excess of nutrients from the soil, but also in creating suitable sites for germination (Fennema 1990, Klooker *et al.* 1999, Jansen 2000, chapter 6).

Restoration of species-rich heaths requires more than the rehabilitation of the abiotic conditions (chapter 6). Biotic factors such as lack of viable seeds and limited seed dispersal may seriously hamper restoration (Bakker & Berendse 1999). As many characteristic species of the species-rich heaths have short-lived seed banks, the re-establishment of the species from seeds is problematic when populations have disappeared (Thompson *et al.* 1997, Bekker *et al.* 1998). Dramatically, dispersal of the characteristic species is also bad (Strykstra *et al.* 1998) and it is likely that many of the original sources in seed dispersal have vanished with the disappearance of the original land use (Poschlod & Bonn 1998). This implies that whenever such characteristic species have disappeared from a reserve, they are very hard to get back.

Furthermore, as the species-rich heaths are rare, habitats are often fragmentated and the populations of characteristic species are usually small. In small populations, processes concerning the genetic variation, demography and reproductive biology are relatively important in the survival of the species. Oostermeyer (1996) has shown the importance of these processes for *Gentiana pneumonanthe*, a species which was once common in species-rich heaths and acidic grasslands but is at present declining in the Netherlands and England (Oostermeyer 1996, based on data by FLORIVON-0 and FLORBASE-1). His model studies showed that, as a result of the small and isolated populations, combined inbreeding and reduced seed production led to a concomitant reduction in population size, regardless of the effects of the abiotic conditions. Moreover, he showed that in *Gentiana pneumonanthe*, the effects of environmental stresses are transferred to the offspring via maternal carry-over effects. Thus, the environment does not only exert its impact on the current population, but its effects may reach into the future.

This implies that, in order to conserve or restore the environment, one should not wait until the environmental stresses have reduced populations to a small number of individuals. Abiotic conditions should be restored as soon as their retardation becomes obvious, hence as soon as soil acidification or nitrogen enrichment become apparent. Early action, when rest populations of characteristic species are still present, has the additional advantage that seed sources are still available.

The conservation of heathland vegetations in the Netherlands is only possible with the aid of appropriate management practices, as the vegetations are semi-natural. At present severe anthropogenic stresses are being enforced on the heathlands, and they make a higher frequency of heathland management and/or restoration measures necessary. One should, however, realise that the effects of these practices are however temporary and that, in general, the biological values of the heathlands can not be fully restored. Therefore, restoration programmes ought to be coupled to a strong policy to reduce the impact of the causes for the heathland decline. A reduction in atmospheric deposition of acidifying and eutrophying pollutants and a reconstruction of the original hydrological systems are nescessary to fully conserve the beauty and value of the heathlands.

CONCLUSION

This thesis has explored the calcicole – calcifuge gradient in heathlands in various ways. Firstly, the existence of such a gradient was studied in a wet heathland. That survey showed that pH-related factors explained the distribution of characteristic species of species-poor and species-rich heathlands. Differences in hydrology caused the existence of the gradient: calcareous seepage water reached the rhizosphere of the species-rich heathland vegetation for a significantly longer period each year than in the species-poor heath. Differences in N and P concentrations were hardly observed in the calcicole – calcifuge gradient on nutrient-poor soils, in contrast to the distribution of species in grass and dwarf-shrub dominated heathlands, in which N-availability is a key factor. Differences in in-situ N-mineralisation or nitrification were neither observed along the calcicole – calcifuge gradient in the wet heathland.

The distribution of plant species along the calcicole-calcifuge gradient at the acid extreme is caused by physiological adaptations to phytotoxic concentrations of aluminium and ammonium, of the species of the species-poor heaths. Characteristic species of the species-rich heathlands, such as *Arnica montana* and *Cirsium dissectum*, lack these adaptations. When grown on nutrient solutions which resemble the soil solution of the species-poor heathlands, *Arnica montana* and *Cirsium dissectum* both severely reduce the uptake of macronutrients as potassium, calcium an magnesium, which may eventually result in shortages of these nutrients. Furthermore, reductions in growth, a poor development of the root system and higher mortality rates have been observed in both plant species. In contrast, *Calluna vulgaris* grew and developed best under the acid conditions, thereby showing to be adapted to its natural habitat.

The lack of adaptations in characteristic species of species-rich heathlands to acid soils (pH < 4.5), makes them sensitive to soil acidification. It is, therefore, conceivable that the decline of species-rich heathlands during the past decades is at least partly due to soil acidification.

The possibilities for restoration of the species-rich heath vegetations were examined in a dry heathland. It was shown that the rehabilitation of the original soil conditions was established by increasing the soil pH to its original value and lowering nutrient concentrations. A combination of liming and sod-cutting was used and proved to be successful. Vegetation development followed the restoration of the abiotic conditions but was not complete, probably due to the absense of viable seeds. It has been concluded that the success of restoration projects depends on the rehabilitation of the abiotic conditions as well as on the availability of seeds or seed sources.

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SAMENVATTING

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DE CALCICOLE – CALCIFUGE GRADIËNT

Planten hebben geen pootjes. Alleen als zaad, en soms met uitlopers, zijn ze in staat zich te verplaatsen. Dan nog bepaalt het toeval doorgaans waar ze terechtkomen. Toch zijn vegetaties of plantengemeenschappen geen verzamelingen van willekeurig ontkiemde en opgegroeide planten, kriskras door elkaar. De omgeving selecteert. Als een standplaats ongeschikt is voor een plant kiemt die daar niet of hij kwijnt weg. Planten die zich ter plaatse wel thuis voelen groeien krachtig uit en concurreren de zwakkeren weg. Zo ontstaan bossen en struwelen, graslanden, ruigtes, rietvelden en purperen heiden, elk op hun 'eigen' plek.

Een verloop in een eigenschap van een terrein, bijvoorbeeld van hoog naar laag, van droog naar nat, of van basisch naar zuur, brengt vaak een overgang in de vegetatie teweeg. Het verloop, ofwel de gradiënt, van neutraal naar zuur in heidelandschappen vormt het onderwerp van dit proefschrift. Deze gradiënt wordt door ecologen aangeduid met de Engelse wetenschappelijke term calcicole-calcifuge gradient.

Calcicole-calcifuge gradiënten lopen van bodems met een hoge pH (de calcicole kant) naar zuurdere terreindelen (het calcifuge uiteinde van de gradiënt). De term verwijst niet alleen naar de zuurgraad van de bodem; dan zou er immers ook gesproken kunnen worden van een zuur-gradiënt of een kalk-gradiënt. De term 'calcicole-calcifuge gradiënt' verwijst tevens naar de planten die op de verschillende delen van de gradiënt groeien en naar de aanpassingen van die planten aan hun milieu. In die zin zou je de term kunnen vertalen als een gradiënt van kalkminnende (calcicole) en kalkmijdende (calcifuge) soorten. Ware het niet dat de karakteristieke plantensoorten van de heide allemaal kalkmijdend zijn. De term kan dan ook maar het beste onvertaald blijven.

DIT PROEFSCHRIFT IN EEN NOTEDOP

Dit proefschrift gaat over de calcicole - calcifuge gradiënt in heide in de breedste zin van het woord. Ik beschrijf het voorkomen van dergelijke gradiënten in een natte heide en de aanpassingen van karakteristieke plantensoorten uit verschillende delen van de gradiënt aan hun milieu. Dit geeft inzicht in het ecologisch functioneren van die gradiënten. En vervolgens is dit inzicht gebruikt om voor een aantal heidetypen die sterk achteruitgaan een passend herstelbeheer te ontwikkelen, dat uitgaat van het herstel van de oorspronkelijke omstandigheden in de heide.

De algemene hypothese die aan dit proefschrift ten grondslag ligt is dat de karakteristieke soorten van soortenrijke heide kunnen worden opgevat als calcicole soorten, plantensoorten die zijn aangepast aan bodems met een vrijwel neutrale pH. Dit in tegenstelling tot de soorten van de soortenarme heide, die als calcifuge plantensoorten worden beschouwd, dus als soorten die kenmerkend zijn voor zure bodems. We gingen er bij aanvang van het onderzoek vanuit dat de calcicole plantensoorten van soortenrijke heide niet zijn aangepast aan erg zure bodems, omdat zij niet bestand zijn tegen de hoge aluminium en/of ammonium gehalten zoals die in zure, soortenarme heide voorkomen. Ook is het mogelijk dat een verstoring in de stikstofvoeding, te veel ammonium in plaats van nitraat, voor problemen zorgt bij calcicole planten. Deze hypothesen zijn door het onderzoek bevestigd.

HEIDE

In principe kan 'heide' op twee manieren worden opgevat: strikt als vegetatiekundige eenheid, dan is het een plantengemeenschap van voornamelijk dwergstruiken zoals struikheide en dopheide. Anderzijds kan de heide worden beschouwd als een landschapstype, waarin meerdere vegetatietypen gevestigd zijn. In Nederland werd de heide vele eeuwen gezien als landschap: van oudsher gebruikte de bevolking het begrip voor alle gemeenschappelijke, extensief begraasde gronden. Dit houdt in dat de heide niet alleen de paarse, uitgestrekte vlakten omvat, maar dat ook graziger stukken met een groter aandeel grassen en kruiden tot de heide werden gerekend. In dit proefschrift ga ik uit van de laatstgenoemde definitie, dus beschouw ik de heide als landschap.

In het heidelandschap komen verschillende plantengemeenschappen voor. Hieraan ten grondslag liggen verschillen in de chemische en fysische omstandigheden in de bovenste laag van de bodem, daar waar de planten wortelen. Deze verschillen bepalen onder andere het voorkomen van calcicole-calcifuge gradiënten.

Alle heidevegetaties komen voor op zure (pH < 6,5), voedselarme gronden. Het vochtgehalte van de bodem speelt een bepalende rol in het voorkomen van 'natte' of 'droge' heidevegetaties. Kleine verschillen in zuurgraad en voedselrijkdom van de bodem (met name de beschikbaarheid van stikstof en fosfaat) tekenen vervolgens voor verdere verschillen in vegetatie. Ten vierde speelt successie een rol; successie is het proces waarbij het ene vegetatietype na verloop van tijd overgaat in een ander, bijvoorbeeld het verschijnsel dat heide zonder ingrepen van de mens in Nederland overgaat in bos.

De meest bekende heides zijn de uitgestrekte paarse heidevelden: deze zijn kenmerkend voor de voedselarmste en zuurste delen van de heide. Op droge bodems bestaan de velden vooral uit struikheide-planten, terwijl op vochtiger plaatsen dopheide de kenmerkende soort is. Deze heides zijn vaak arm aan plantensoorten en alhoewel er in vegetatiekundig opzicht meerdere typen in te onderscheiden zijn, worden zij in dit proefschrift samengevat met de term 'soortenarme heide'.

Op overgangen naar iets minder zure plaatsen, naar voedselrijkere stukken of op overgangen naar hoogvenen worden vaak vegetatietypen aangetroffen die veel rijker zijn aan kruiden. Valkruid, liggende vleugeltjesbloem, blauwe knoop, klokjesgentiaan, heidekartelblad, beenbreek en de gevlekte orchis zijn voorbeelden van soorten die je op dergelijke plaatsen kunt vinden. En hoewel deze plantensoorten kenmerkend zijn voor een aantal verschillende vegetatietypen, worden zij in dit proefschrift samengenomen onder één noemer: 'soortenrijke heide'.

DE CALCICOLE-CALCIFUGE GRADIËNT IN HEIDE

Al lang is bekend dat het voorkomen van soortenarme en soortenrijke heide gerelateerd is aan de zuurgraad van de bodem. In de internationale literatuur spreekt men dan ook van de calcicole-calcifuge gradiënt. De gradiënt strekt zich in heide uit van zeer zure bodems ($pH < 4{,}5$) via een middentraject van zwak zure bodems ($4{,}5 < pH < 6{,}5$) tot circumneutrale bodems (pH ca. 7). Hieraan ten grondslag liggen verschillende buffermechanismen in de bodem, processen in de bodem waardoor de zuurgraad vrijwel constant blijft.

Bodems worden gebufferd door verschillende mechanismen, die kenmerkend zijn voor de hierboven genoemde typen bodem. De buffermechanismen zijn afhankelijk van de beschikbare stoffen in de bodem. Zo worden circumneutrale bodems voornamelijk gebufferd doordat carbonaten (kalk) in oplossing gaan wanneer zij met zuur in contact komen. Hierbij komen o.a. calcium en magnesium vrij. De zwak zure bodems van het middentraject ($4{,}5 < pH < 6{,}5$) worden voornamelijk gebufferd doordat zuurionen aan het bodemadsorptiecomplex worden uitgewisseld tegen de zogeheten basische kationen calcium, magnesium, kalium en natrium (men noemt dit ook wel de kationuitwisselingsbufferrange). Deze basische kationen zijn belangrijke voedingsstoffen voor planten. Wanneer deze niet meer geadsorbeerd zijn aan het bodemadsorptiecomplex, kunnen zij uitspoelen naar diepere bodemlagen, waar onbereikbaar zijn voor planten.

In de meest zure bodems is de beschikbaarheid van deze voedingsstoffen voor planten dan ook laag. Daar komt bij dat deze bodems voornamelijk worden gebufferd door aluminium(hydr)oxiden, waarbij aluminium (Al^{3+}) uiteindelijk vrij in de bodem komt. Dit vrije aluminium is voor veel planten schadelijk. Daarnaast bevatten de zure bodems vaak hoge concentraties zuurionen, ijzer, mangaan en ammonium, wat eveneens giftig kan zijn voor planten.

DE CALCICOLE - CALCIFUGE GRADIËNT IN HET BREKLENKAMPSE VELD

Hoofdstuk 2 beschrijft een gedetailleerde studie van de calcicole – calcifuge gradient in natte heide. Dit onderzoek is uitgevoerd in het Breklenkampse veld, een heidegebied in Noordoost Twente, waar de oorspronkelijke heidevegetatie nog intact is. In dit terrein is in april 1995 een proefstrook uitgezet over twee heidevegetaties: een soortenarme heide, waarin dopheide, struikheide en pijpestrootje de meest karakteristiek soorten waren, en een soortenrijke heide. In deze laatste waren blauwe knoop, blauwe zegge en heidekartelblad aspectbepalend. De zones lagen zo'n 8 m. uit elkaar, maar de soortenarme zone lag gemiddeld 11 cm hoger dan de soortenrijke zone. Doordat de vegetatiezones zo dicht bij elkaar lagen, kan er vanuit worden gegaan dat de toevoer van stikstofvoeding en zuurionen uit de lucht voor beide zones vergelijkbaar was.

Het bleek dat het voorkomen van soortenarme en soortenrijke heide met name werd bepaald door de pH van de bodem en de daaraan gerelateerde factoren zoals de calcium, magnesium en aluminiumconcentratie in de wortelzone. Met recht kan dus gesproken worden van een calcicole – calcifuge gradiënt. Deze gradiënt vindt zijn oorsprong in verschillen in de grondwaterhuishouding: in de soortenrijke heide kwam het grondwater in de periode 1991 – 1995 gemiddeld 121 dagen in de wortelzone. In de soortenarme zone slechts 46 dagen. Dit grondwater is er de oorzaak van dat de soortenrijke heidezone voornamelijk wordt gebufferd door kationuitwisselingsprocessen, terwijl de soortenarme zone wordt gebufferd door het oplossen van aluminium(hydr)oxiden.

De verschillen in buffermechanismen leiden tot een heel andere samenstelling van het bodemvocht in de wortelzone: in de soortenarme zone worden veel hogere concentraties aluminium gemeten, wat giftig kan zijn voor veel planten. Daarnaast zijn de concentraties van belangrijke voedingsstoffen magnesium en calcium lager: ook dit zal specifieke aanpassingen van de planten in de soortenarme zone vragen.

Opmerkelijk genoeg bleken er nauwelijks verschillen te zijn in de beschikbaarheid van de voornaamste voedingsstoffen voor planten, te weten stikstof en fosfaat. Daarbij werden er evenmin verschillen gemeten in de snelheid waarmee stikstofmineralisatie en nitrificatie verliepen, de processen waardoor stikstof wordt omgezet in ammonium en nitraat, vormen die planten kunnen opnemen. Dit is bijzonder, omdat uit andere onderzoeken bekend is dat deze processen sterk door de pH van de bodem en door de samenstelling van de plantengemeenschappen worden beïnvloed. En deze factoren verschillen in de beide zones aanmerkelijk. Ik veronderstel dat het intensieve maaibeheer dat in het Breklenkampse Veld wordt gevoerd, debet is aan het ontbreken van verschillen in stikstofmineralisatie en nitrificatie.

AANPASSINGEN VAN PLANTEN AAN DE CALCICOLE – CALCIFUGE GRADIËNT

Vervolgens gaat het proefschrift in op de aanpassingen van heidesoorten aan hun milieu. Gekoppeld hieraan was de vraag waarom karakteristieke soorten van soortenrijke heide níet en planten van soortenarme heide wél in het veld worden aangetroffen bij een pH lager dan 4,5.

Dit gebeurde met behulp van fysiologische experimenten in het laboratorium, waarbij kiemplantjes op hydrocultures werden opgekweekt onder verschillende omstandigheden die gerelateerd zijn aan de zuurgraad van de bodem. De pH zelf werd niet gevarieerd, omdat uit soortgelijk onderzoek in bossen bekend was dat de aluminiumconcentratie en de vorm waarin planten stikstof opnemen (ammonium of nitraat) belangrijker zijn dan de pH zelf. In deze experimenten werd gebruik gemaakt van drie soorten: struikheide, als kenmerkende soort van soortenarme heide, valkruid en spaanse ruiter. De laatste twee soorten zijn karakteristiek voor soortenrijke heidevegetaties, waarbij spaanse ruiter vooral voorkomt in natte en valkruid in droge en vochtige heiden.

Dit type onderzoek biedt de mogelijkheid om aanpassingen van planten aan een bepaalde stressfactor, bv. een hoge aluminiumconcentratie, nader te bestuderen, zonder daarbij gehinderd te worden door andere factoren die ook verschillen maar waarin je nu even niet geïnteresseerd bent (bijvoorbeeld het vochtgehalte van de bodem). Om de relatie van de fysiologische experimenten met het veld zo realistisch mogelijk te houden werden de planten opgekweekt op voedingsstoffenoplossingen die in samenstelling veel lijken op het natuurlijke bodemvocht.

Stikstofvoeding: ammonium of nitraat?

Eerst wordt ingegaan op het effect van verschillende stikstofvormen, ammonium en nitraat, op de drie planten. Onder invloed van verzuring vindt meestal een remming van de nitrificatie plaats, waardoor in zure bodems ammonium de meest voorkomende stikstofvorm is. In meer gebufferde bodems komt voornamelijk nitraat voor. Beide vormen van stikstof kunnen door planten worden opgenomen, maar veel plantensoorten nemen voornamelijk de vorm op die in hun milieu het meest voorkomt. In zure bodems is dit ammonium, in neutrale bodems nitraat of een mengsel van ammonium en nitraat. Daarnaast is van ammonium bekend dat het in hoge concentraties giftig is voor sommige plantensoorten.

Beide principes gaan ook op voor de onderzochte heideplanten. Struikheide, de soort van de soortenarme, zure heide ontwikkelt zich het best op ammonium, terwijl zowel spaanse ruiter als valkruid zich beter ontwikkelen op nitraat. Beide planten vertonen vergiftigingsverschijnselen wanneer het ammoniumgehalte in de voedingsstoffenoplossing boven de 100 μmol l^{-1} stijgt: de zijwortels groeien niet uit en de bladeren worden geel. Ook blijft de wortelgroei achter bij de groei van de bladeren, waardoor de plant extra kwetsbaar wordt voor uitdroging. Immers, door het grote bladoppervlak verdampt zo’n plant meer, terwijl de plant minder capaciteit heeft om de watertekorten aan te vullen.

Met toenemende ammoniumconcentratie in de voedingsstoffenoplossing blijven de planten kleiner. Tevens gaan er meer planten dood naarmate de ammoniumconcentratie in de voedingsstoffenoplossing stijgt. Dit is met name het geval bij valkruid; de sterfte van spaanse ruiter en struikheide neemt niet toe wanneer er meer ammonium wordt toegediend. Spaanse ruiter blijkt wel extra gevoelig te zijn voor een eenzijdig ammoniumaanbod: ammonium alleen is veel giftiger dan wanneer het in combinatie met nitraat wordt aangeboden aan de planten.

Aluminium

Wanneer de planten worden opgekweekt op een reeks oplossingen met toenemende aluminiumconcentraties, dan geeft dit soortgelijke resultaten als een toename in de ammoniumconcentratie. Struikheide is grotendeels ongevoelig voor aluminium, terwijl de karakteristieke soorten van de soortenrijke heide slechter groeien naarmate de aluminiumconcentratie hoger is. Opnieuw blijkt het wortelstelsel van valkruid en spaanse ruiter zich slecht te ontwikkelen bij hoge aluminiumconcentratie (tot 500 μmol l^{-1}), en worden de planten geel. Echter, het negatieve effect van aluminium kan deels teniet gedaan worden door een hogere calciumcontratie: hieruit blijkt dat niet alleen de absolute concentratie aluminium van belang is, maar dat ook de verhouding tussen aluminium en calcium in het bodemvocht de toxiciteit van aluminium bepaalt.

Stikstofvorm en aluminium

Als derde is een experiment uitgevoerd met spaanse ruiter en valkruid, waarbij zowel de aluminiumconcentratie als de stikstofvorm werden gevarieerd. De stikstofconcentratie werd steeds laag gehouden (100 μmol l^{-1}). De filosofie achter dit experiment was dat bij bodemverzuring enerzijds de aluminiumconcentratie stijgt en anderzijds de dominante stikstofvorm verandert van nitraat in ammonium. Echter, vrijwel alle in de literatuur beschreven experimenten over aluminiumtoxiciteit en ook het zojuist beschreven experiment, werden uitgevoerd met nitraat als stikstofbron. Het is daarentegen realistischer om de effecten van hoge aluminiumconcentraties te bestuderen met ammonium of een mengsel van ammonium en nitraat als stikstofbron. Een dergelijke proefopzet geeft tevens een aanwijzing van wat de belangrijkste reden van achteruitgang voor de planten is: toxiciteit van ammonium of van aluminium.

De resultaten van dit experiment komen grotendeels overeen met de resultaten van de eerdere proeven. Wel wordt duidelijk dat het effect van stikstofvoeding het effect van aluminium overtreft: ammonium, zelfs bij lage concentraties, is veel giftiger dan aluminium. Met name spaanse ruiter blijkt erg intolerant te zijn voor een eenzijdig ammoniumaanbod. Valkruid daarentegen, groeit het best op een mengsel van ammonium en nitraat, zij het dat de ammoniumconcentratie laag moet blijven. Desalniettemin hebben we bij valkruid wel vergiftigingsverschijnselen door ammonium waargenomen, bijvoorbeeld een verstoorde wortelgroei.

Interacties tussen aluminium en stikstofvorm zijn in valkruid zelden waargenomen. Bij spaanse ruiter was dit wel het geval, met name waar het de lengte van het wortelstelsel, de specifieke wortellengte, de verhouding tussen wortel en spruit en de opname van voedingsstoffen betrof. Over het algemeen kan men stellen dat het negatieve effect van aluminium veel groter is wanneer het medium waarop de planten zijn opgekweekt ammonium bevat dan wanneer de planten alleen op nitraat worden opgekweekt.

Effecten van ammonium en aluminium op de opname van voedingsstoffen

In alle drie de fysiologische experimenten is behalve naar groei en ontwikkeling van de planten, ook gekeken naar de opname van nutriënten. Met name spaanse ruiter en valkruid blijken slecht in staat calcium, magnesium en kalium op te nemen uit een voedingsstoffenmengsel waarin ook ammonium en/of aluminium aanwezig is. Calcium, magnesium en kalium zijn echter belangrijke voedingsstoffen voor een plant en het is niet onwaarschijnlijk dat er tekorten aan deze nutriënten ontstaan onder invloed van hoge aluminium- en ammoniumconcentraties. Ook struikheide vermindert de opname van calcium bij een toenemende ammoniumconcentratie, maar dit gaat niet gepaard met een vertraagde groei of met andere toxiciteitsverschijnselen. Kennelijk kunnen de planten nog voldoende calcium opnemen. In geval van bodemverzuring in het veld komt daarbij dat deze zogeheten basische kationen sowieso al veel minder beschikbaar zijn onder zure omstandigheden, hetgeen tekorten aan deze voedingsstoffen extra in de hand zal werken.

Ook blijkt dat valkruid bij de opname van ammonium zuurionen uitstoot, zodat de wortelzone zuurder wordt. Hierdoor zullen kalium, calcium en magnesium verder uitspoelen en zal de aluminiumconcentratie in de wortelzone verder stijgen. Met andere woorden, het negatieve effect van ammonium wordt verder versterkt.

Spaanse ruiter daarentegen stoot geen zuurionen uit wanneer hij ammonium opneemt onder zure omstandigheden, maar dit kan nauwelijks een voordeel worden genoemd: omdat de opname van ammonium gepaard gaat met H^+-productie, en dit zuur niet wordt uitgescheiden, verzuren de cellen in de wortel. Dit is waarschijnlijk de oorzaak voor de sterke toxiciteit van ammonium voor deze soort.

Op zure heidebodems ($pH < 4,5$) zullen de soorten van soortenrijke heiden dan ook ernstig in de problemen komen: door de toename van de concentraties ammonium en aluminium in de bodem raakt de wortelgroei verstoord en wordt de opname van o.a. kalium, calcium en magnesium verminderd. Daarbij komt dat doordat de spruit van de planten op ammoniumvoeding harder groeit dan het wortelstelsel, de planten sneller verwelken wanneer de bodem uitdroogt. Dit is aangetoond voor valkruid.

Het is niet zomaar mogelijk om de resultaten van hydrocultuurexperimenten door te trekken naar de veldsituatie. Het hierboven geschetste beeld van wat er gebeurt met niet-aangepaste planten op zure bodems is een soort worst-case scenario. Verdere experimenten zullen moeten uitwijzen in hoeverre dit ook daadwerkelijk optreedt in het veld. Daar staat echter tegenover dat de resultaten van de hydrokultuurexperimenten opvallend goed overeenstemmen met die van veldonderzoeken in heide, hetgeen de achteruitgang van de soortenrijke heide door verzuring zeer aannemelijk maakt.

HERSTEL VAN SOORTENRIJKE HEIDE

Soortenrijke heiden behoren tot de meest diverse oecosystemen van ons land. In de twintigste eeuw zijn deze oecosystemen sterk in oppervlakte en in kwaliteit achteruitgegaan in ons en de ons omringende landen. De laatste decennia zijn verzuring, vermesting en verdroging belangrijke oorzaken van die achteruitgang. Dit alles maakt herstelprojecten die specifiek gericht zijn op de restauratie van deze typen heide zeer gewenst.

Herstelprojecten hebben de meeste kans van slagen wanneer de herstelmaatregelen aansluiten bij de oorzaak van de achteruitgang van het oecosysteem. Met andere woorden, in verzuurde heide moet je de verzuring bestrijden, in vermeste heide de vermesting. In het natuurgebied de Schaopedobbe in Friesland werd in 1990 een herstelproject uitgevoerd volgens dit principe. De herstelmaatregelen hadden tot doel de volgende karakteristieke heidevegetaties te herstellen:

1. soortenrijke heide in een verzuurde heide. Dit deel van het terrein is niet geëutrofieerd;
2. soortenrijke heide op een stuk van het terrein dat in het verleden een aantal jaar als akker is gebruikt en in die tijd is bekalkt en bemest. Dit deel van het terrein is te voedselrijk voor de soortenrijke heide maar heeft wel de goede zuurgraad;
3. een soortenarme heide op een vergrassend deel van het terrein. Hier heeft waarschijnlijk nooit soortenrijke heide gestaan.

Om te bestuderen welke maatregelen het meest succesvol zijn om de beoogde heidevegetaties te herstellen, werden drie proefvelden uitgezet. In ieder proefveld werden combinaties van plaggen (ter bestrijding van de eutrofiëring) en bekalking (om de verzuurde bodem te herstellen) toegepast. Voor de bekalking werden verschillende materialen gebruikt (mergel, dolokal, gips en lavameel) in verschillende doses, die steeds tot doel hadden de zuurgraad van de bodem (terug) te brengen in de kationuitwisselingsbufferrange (pH 4,5 – 6).

Het bleek dat de gewenste bodemcondities zonder problemen hersteld konden worden: de bodem werd minder zuur als gevolg van de bekalkingen, en de calcium- en magnesiumgehalten stegen (alleen in de dolokalbehandelingen). Daarentegen daalde het aluminiumgehalte. Plaggen verminderde de gehalten aan de voornaamste voedingsstoffen, stikstof en fosfaat, aanzienlijk. Met andere woorden, het is mogelijk om met uitgekiende herstelmaatregelen opnieuw de voedselarme, zure tot licht gebufferde bodems te creëren die karakteristiek zijn voor soortenrijke heide.

De effecten van de maatregelen op de vegetatie zijn divers, maar in het algemeen positief. Vijf jaar nadat de maatregelen zijn genomen heeft zich op de geplagde delen van de geëutrofieerde, vergraste heide een soortenarme heide gevormd, waarin de bedekking van de grassen lager is dan voordat er geplagd werd. Struikheide heeft zich wel volop gevestigd, maar een andere karakteristieke heidesoort, kraaiheide, keerde na plaggen (nog) niet terug.

Op de andere twee proefvelden is een duidelijk herstel van de soortenrijke heide te zien: met name in de geplagde, bekalkte delen van de verzuurde soortenrijke heide. De vegetatieontwikkeling op de geplagde delen van het voormalige akkertje verloopt eveneens gunstig. De bekalkingen lijken hier echter geen toegevoegde waarde te hebben omdat de bodem van dit terrein zich al in de goede bufferrange bevond. In beide proefvelden waar de vestiging van een soortenrijke heide werd beoogd blijken zich een aantal karakteristieke planten van deze gemeenschappen te vestigen.

De respons van valkruid op bekalking is ook meer in detail onderzocht. In de bestaande, achteruitgaande populatie, leidde bekalking tot een toename in het aantal planten, terwijl er in de onbekalkte delen van het proefveld een achteruitgang te zien was. Daarnaast bleken de planten meer zaad te vormen in de bekalkte dan in de onbekalkte delen. Op de geplagde delen van de proefvelden, kiemde en groeide valkruid aanzienlijk beter wanneer er was bekalkt dan wanneer dit niet het geval was. Ook de overleving van de planten was beter na bekalking.

Kortom, herstel van heidevegetaties lijkt mogelijk, mits je de goede maatregelen gebruikt. Hieraan zit echter een maar: op geen van de proefvelden keerden soorten terug die niet in de directe nabijheid van de proefvelden groeiden. Daardoor ontbreken, met name in de soortenrijke heidevegetaties, een aantal karakteristieke plantensoorten die er op basis van de bodemcondities wel zouden kunnen worden verwacht. Dit maakt duidelijk dat herstel van vegetaties niet alleen afhankelijk is van herstel van de abiotische omstandigheden. Restauratie begint weliswaar met herstel van de abiotische condities, maar vervolgens zijn de zaadvoorraad in de bodem en/of de mogelijkheid om via verspreiding vanuit de directe omgeving een ‘hersteld’ terrein te koloniseren bepalend of een soortenrijke heide zich uiteindelijk daadwerkelijk herstelt. In veel herstelprojecten zal de aan- of afwezigheid van zaad van de karakteristieke soorten dan ook sterk bepalend zijn voor het uiteindelijke succes van de projecten, want planten hebben nu eenmaal geen pootjes.

NAWOORD

Een balletje kan raar rollen, een kleine familiegeschiedenis kent grote contrasten. Mijn grootouders hebben met veel moeite de Brabantse heide bij Baarschot en Haghorst ontgonnen. Oma van Bijsterveldt schrijft daarover in haar ongepubliceerde levensverhaal: '*De eerste zondag hebben we over de boerderij gewandeld, maar we* (mijn opa en oma) *liepen elkaar verloren tussen de hoge bulten en diepe kuilen die we tegen kwamen. Broer Jan was al een paar jaar bezig de grond te effenen*'. Dit was in 1932 en de heide moet er toen dus ongeveer zo uit hebben gezien. De te ontginnen heide moest met '*paard en maalbord, zoals men het noemde, men zou het ook een grote schep kunnen noemen, geëgaliseerd worden. Een bijzonderheid zou ik noemen dat de diepte van zo'n gat zoo was, dat toen mijn man eens een halfwas berkenboom inplantte alleen de top te zien was*' (Oma van Bijsterveldt). Een paar jaar later werd het bedrijf verder uitgebreid: '*Er lag in de buurt nog een stuk heide met een ven erbij en dat werd bijgekocht voor de prijs van F 150,- per ha.*' Ruim zeventig jaar later promoveert hun kleindochter op een onderzoek dat is begonnen om methoden te ontwikkelen om de laatste resten van de heide te behouden.

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Maaike

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CURRICULUM VITAE

Maaike de Graaf is geboren op zondagochtend, 14 februari 1965 te Tilburg. In 1983 sloot zij de middelbare school (het Dr. Mollercollege te Waalwijk) af met een atheneum-beta diploma. Datzelfde jaar begon Maaike met haar biologiestudie aan de Katholieke Universiteit van Nijmegen. In de afstudeerfase specialiseerde zij zich in de plantenoecologie, de bodemmicrobiologie en de voorlichtingskunde.

Na het behalen van het doctoraalexamen in 1989 werd zij voor korte tijd aangesteld als medewerker van de afdeling Plantenoecologie van de KUN, om in opdracht van Rijkswaterstaat een rapport te schrijven over vegetatiezonering in de uiterwaarden van de Waal, IJssel en Nederrijn. In 1990 werd zij aangesteldbij de Afdeling Aquatische Oecologie van dezelfde universiteit om de achteruitgang van soortenrijke heide in Nederland te onderzoeken en nieuwe beheersmethoden te ontwikkelen om het behoud van deze oecosystemen te waarborgen. Dit gebeurde in het kader van het project Effectgerichte Maatregelen tegen verzuring en eutrofiëring van matig mineraalrijke heide en schraallanden, in opdracht van het Ministerie van Landbouw, Natuurbeheer en Visserij. Het is dit project dat de basis vormde voor dit proefschrift. Sinds 1998 is Maaike als ecologe verbonden aan de adviesgroep Water van Grontmij Advies & Techniek.