

Linkages of Nitrogen and Phosphorus Availability to Ecosystem Processes and Succession in Forests of Northern Sweden and New Zealand

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Abstract

Initially after catastrophic disturbance ecosystems undergo a build-up phase but in the long term absence of catastrophic disturbance ecosystems can enter a phase of decline called ecosystem retrogression. It is characterised by reduced productivity, decomposition rates and rates of nutrient cycling as a consequence of nutrient limitation. Over the course of long-term soil development, phosphorus (P) availability decreases due to leaching and chemical immobilisation, leading to increased soil N: P ratios during retrogression. In contrast, nitrogen (N) is continuously supplied to natural ecosystems through biological N₂ fixation, though late successional plant species can have a negative effect on N availability during retrogression, reinforcing N limitation. I studied variation in supply and availability of N and P throughout a 5000 year retrogressive chronosequence in which the soil N: P ratio increases, and investigated how it affected the nutrient status of soil microbes and plants. I found that total N increases considerably during retrogression as a consequence of increased N₂ fixation. Total P did not change across the chronosequence but labile mineral P declined in the retrogressive stage and this was connected to decreased soil microbial activity. Plant nutrient status showed diverging trends between trees and dwarf shrubs across the chronosequence, indicating increasing resource partitioning as retrogression proceeds. Variation in leaf traits was due to shifting species composition rather than within species changes. I also studied factors influencing the availability of N and P during the build-up phase of succession as affected by an important extrinsic driver, i.e., herbivory. Herbivory of a N₂ fixing shrub had considerable negative effects on the nutrient status of not only its own growth and nutrient status, but also that of neighbouring non-fixing plants and soil processes. These studies in combination have demonstrated how changing availability of nutrients during succession, both in the build-up phase and the retrogressive phase, can be driven by biological processes such as N₂ fixation and species effects on carbon (C) quality and, how this in turn leads to differences in the relative success of coexisting plant species in the community. As such, these results demonstrate the dynamic and variable nature of nutrient limitation and the processes leading to it.

Keywords: Nitrogen, phosphorus, succession, wildfire, retrogression, boreal forest, N₂ fixation, N: P ratio, herbivory

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Contents

List of Publications	7
1 Introduction	9
1.1 Nutrient limitation of ecosystems	9
1.2 Ecological stoichiometry	11
1.3 Supply and availability of N and P through ecosystem succession	13
1.4 Effect of herbivory on N and P supply and availability	15
1.5 Objectives	16
2 Material and methods	19
2.1. Study systems	19
2.2. Experimental design and sampling	21
2.3. Methodological aspects	23
2.3.1 Nitrogen fixation measured with acetylene reduction	23
2.3.2 Microbial respiration measurements	23
2.3.3 Sequential extraction of phosphorus	24
2.3.4 Plant traits and weighted averages	24
2.4. Statistical evaluation	35
3. Results and discussion	27
3.1. Input, supply and availability of N	27
3.2. Supply and availability of P	29
3.3. Microbial nutrient limitation and N: P stoichiometry	30
3.4. Plant nutrient limitation and N: P stoichiometry	32
3.5. Effect of herbivory on the nutrient status and interactions of plants	35
3.5. Conclusions	37
References	41
Acknowledgements	50

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Lagerström A, Nilsson M-C, Zackrisson O, Wardle DA (2007). Ecosystem input of nitrogen through biological fixation in feather mosses during ecosystem retrogression. *Functional Ecology* 21, 1027-1033.
- II Lagerström A, Esberg C, Giesler R, Wardle DA (2009). Soil phosphorus and microbial response to a long-term wildfire chronosequence in northern Sweden. *Biogeochemistry* 95, 199-213.
- III Lagerström A, Nilsson M-C, Wardle DA. Response of leaf and litter nutrients, and nutrient resorption, to ecosystem retrogression on boreal forested islands in northern Sweden (manuscript).
- IV Lagerström A, Bellingham PJ, Bonner KI, Wardle DA. The effect of simulated herbivory on growth and nutrient status of focal and neighbouring plants (manuscript).

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1 Introduction

1.1 Nutrient limitation of ecosystems

Availability of nutrients constrains primary productivity of forests worldwide. The most commonly limiting nutrients for plant growth are nitrogen (N) and phosphorus (P) (Grime 1979, Aerts and Chapin 2000). Phosphorus is derived from weathering of bedrock and its supply is therefore constrained largely by the amounts present in parent material, while N input can be replenished through biological N₂ fixation. Theory suggests that as a consequence, P availability regulates the availability of other nutrients, and supply of N (and other nutrients) and is adjusted through biological feedbacks to match P in adequate proportions, meaning that P availability is the major determinant of the productivity of ecosystems worldwide (Redfield 1958, Cole and Heil 1981, Coleman et al. 1983). Availability of nutrients (notably N and P), and consequently productivity, changes gradually throughout the course of ecosystem succession (Odum 1969, Walker and del Moral 2003). Though the literature on succession is extensive, the understanding of the importance of nutrient stoichiometry driving succession in different ecosystems is incomplete (but see Walker et al. 1983, Vitousek 2004, Wardle et al. 2004a). The overall focus of this thesis is how the above- and belowground parts of the ecosystem interact to control availability of major nutrients (notably N and P) through a range of processes summarized in Fig. 1, and how they in turn regulate ecosystem processes during different stages of succession. This knowledge is vital for

understanding the regulation of ecosystem processes in forest ecosystems, and for long-term forest management and conservation.

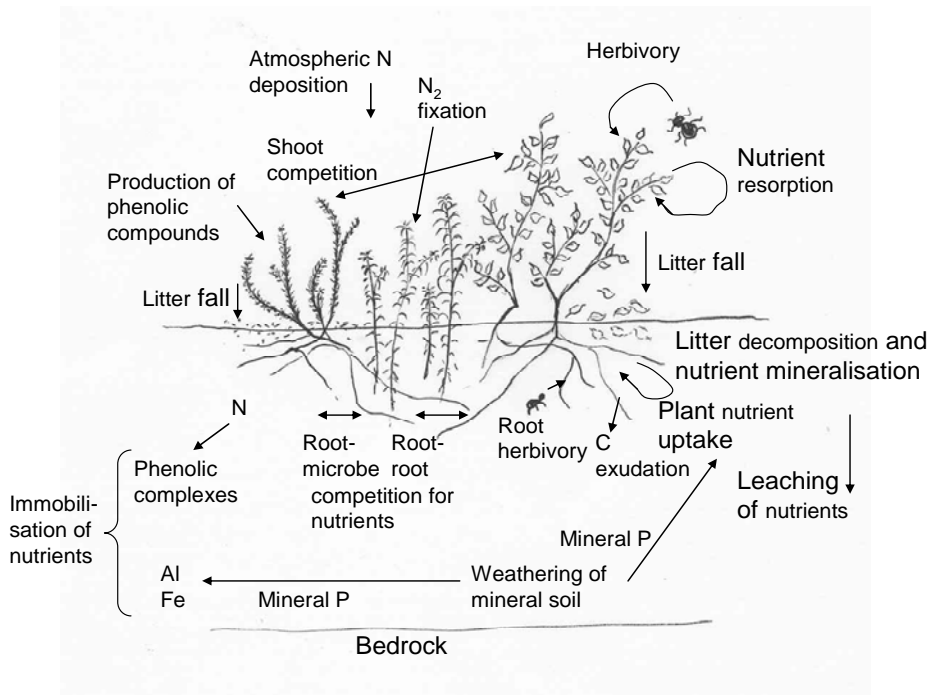


Figure 1. Overview of aboveground and belowground processes that control the supply and availability of N and P in ecosystems. Phosphorus is derived from mineralisation of the bedrock and over time becomes occluded or lost through leaching. Nitrogen is derived from biological N_2 fixation and atmospheric deposition, and can become immobilised in phenolic complexes. Plants regulate their nutrient losses through litter fall and resorption, but also through competition for nutrients with other plants and microbes. Herbivory can trigger nutrient mineralisation through inducing exudation of carbon to the rhizosphere, which can benefit both the target plant and the surrounding plant and microbial community.

1.2 Ecological stoichiometry

Ecological stoichiometry builds on the fact that life is made up of chemical reactions, which involve elements in specific proportions. For example, N is a vital component of the enzyme Rubisco, which catalyses photosynthesis, while P is a building block of RNA in the synthesis of proteins from DNA (Sterner and Elser 2002). The abundance and availability of elements, notably nitrogen (N) and phosphorus (P), can therefore regulate biological processes from the molecular level to the ecosystem level (Elser et al. 2000). The chemical composition of individual organisms directly affects organism growth, reproductive ability, palatability, decomposability and competitive interactions, and thereby indirectly ecosystem processes such as nutrient cycling and productivity (Sterner and Elser 2002). The balance of available elements can therefore have a significant impact on the functioning of whole ecosystems. Ecological stoichiometry is defined by Sterner and Elser (2002) as “The balance of multiple chemical substances in ecological interactions and processes, or the study of this balance.” Redfield (1958) discovered that the elemental composition of marine plankton was the same as that of the dissolved nutrients in their living environment, i.e. $C_{106} : N_{16} : P_1$, now known as “the Redfield ratio”. Redfield proposed that this ratio was the optimal balance of elements for biological processes. He suggested that the ratio of N: P is maintained through internal regulation where N supply is adjusted through biological feedbacks to balance the existing P supply. Hence, P supply determines the productivity of ecosystems (Cole and Heil 1981, Elser et al. 2002). Deviations from the Redfield ratio indicate ecosystem-level nutrient limitation of either N ($N: P < 16$) or P ($N: P > 16$). The Redfield ratio has been central to the study of nutrient dynamics and biogeochemistry of the aquatic ecosystems and is increasingly used in the study of terrestrial ecosystems (Sterner and Elser 2002).

The ratio of leaf N: P concentration is increasingly used to study nutrient limitation in plants (Koerselman and Meuleman 1996, Tessier and Raynal 2003, Güsewell 2004, MacGroddy et al. 2004). The use of N: P ratios to measure plant nutrient limitation is not only less laborious (Koerselman and Meuleman 1996), it also better reflects “the gradual and dynamic character of nutrient limitation” than do fertilization experiments which have traditionally been used to study nutrient limitation in terrestrial ecosystems (Güsewell 2004). Using Redfield-like critical N: P ratios makes it possible to compare nutrient limitation at the plant community level with nutrient

limitation of functional groups and species within the community (Aerts and Chapin 2000). The ratio of nutrients required for maximal growth is similar for most plant species (Knecht and Göransson 2004, Ågren 2008), and though terrestrial plants have more variable element stoichiometry than marine organisms, confined critical N: P ratios of plant biomass exist at the biome level (Table 1; McGroddy et al. 2004, Reich and Oleksyn 2004). Critical Redfield-like ratios for terrestrial plants tend to be near double that of the Redfield ratio (Table 1) and are usually given as mass ratios (as opposed to molar ratios). Recent evidence suggests that Redfield-like critical N: P ratios also exist for soil microbial biomass, and they are nearly identical to the Redfield ratio (Cleveland and Liptzin 2007; Table 1). Measuring the N: P ratio of microbial biomass in addition to plant N: P ratio can give a more complete picture of the nature of nutrient limitation at an ecosystem level (Cleveland and Liptzin 2007), though this has been little explored (but see Paper II).

Table 1. Redfield-like critical N: P ratios of plant foliage, microbial biomass and soil. Both molar (atomic) ratios and their corresponding ratios based on mass are shown. The critical N: P ratio indicates the limit for when organisms are limited by N (below the critical ratio) or P (above the critical ratio).

	Critical N: P ratios		<i>References</i>
	<i>molar</i>	<i>mass</i>	
The Redfield Ratio	16	35	Redfield (1958)
<i>Plant foliage</i>			
Wetlands	6.5-7.5	14-16	Koerselman and Meuleman (1996)
Vegetation in general	5-9	10-20	Güsewell (2004)
Tropical forest	43	92	McGroddy et al. (2004)
Temperate broadleaf forest	28	60	McGroddy et al. (2004)
Temperate conifer forest	22	47	McGroddy et al. (2004)
<i>Soil microbial biomass</i>			
Forest	9	19	Cleveland and Liptzin (2007)
Grassland	5	11	Cleveland and Liptzin (2007)
<i>Soil (total pools)</i>			
Forest	15	32	Cleveland and Liptzin (2007)
Grassland	12	26	Cleveland and Liptzin (2007)

1.3 Supply and availability of N and P through ecosystem succession

The study of long-term ecosystem succession (for definition see Box 1) shows how availability of N and P is linked to the functioning of ecosystems through affecting ecosystem process rates. In early stages of primary succession, i.e. relatively recently after a major disturbance such as glacial retreat, land uplift or volcanic eruption, N is usually the main limiting nutrient. This is because mineral P is readily available through weathering of bedrock, while N availability yet has to build up through nitrogen fixation and atmospheric deposition as soils develop (Crocker and Major 1955, Chapin et al. 1994, Vitousek et al. 2004). In mid-stages of ecosystem succession (often referred to as stable state or equilibrium), N and P are often co-limiting and biomass production is at its maximum. However, after several thousand (or more) years of soil development, P availability will decline as a result of leaching and geochemical immobilization (Walker and Syers 1976, Crews et al. 1995, Turner et al. 2007). In contrast, N can continue to be supplied through biological N₂ fixation in very late stages of ecosystem succession (Vogel and Gower 1998, Crews et al. 2000, Perez et al. 2004, Zackrisson et al. 2004, Smithwick 2005, Menge and Hedin 2009, Mitchell and Ruess 2009; see also Paper I). However, because biological organisms require nutrients in certain stoichiometric proportions, the lack of P leads to severe nutrient limitation, causing these old ecosystems to enter a stage of decline (retrogression) (Walker and Syers 1976, Walker et al. 2001). Retrogression characteristically involves declining productivity, decomposition rate and rate of nutrient cycling, in some cases to the extent that the soils can no longer support forest growth (Walker et al. 2001, Vitousek et al. 2004, Wardle et al. 2004a).

Retrogression has been demonstrated for natural forests that have experienced several thousand years without disturbance, ranging from subtropical to temperate and boreal zones (Wardle et al. 2004a). Though declining P-availability causes retrogression in many studied retrogressive chronosequences, there is also some evidence of increasing N limitation during retrogression in chronosequences involving land uplift (Northup et al. 1995) and increasing time after wildfire (Wardle et al. 1997, 2003, 2004a, Lecomte et al. 2006). Retrogression involving N limitation is mainly driven by biological processes such as plant-soil feedbacks. The advancement of succession promotes dominance of species that produce low quality litter with high concentrations of polyphenolic compounds, which immobilize humus N and impair nutrient cycling and availability (Northup et al. 1995,

Wardle et al. 1997, 1998). These processes are usually also linked to declining soil pH, which can negatively affect the availability of both N and P (Giesler et al. 1998). The relative importance of P availability in promoting retrogression in mainly N-limited ecosystems is unclear, but evidence from an N-limited boreal forest shows a significant reduction of P availability due to intense mineralisation by fungi already at a soil age of 2 000 years (Hoffland et al. 2002). In addition, the development of very deep humus layers in the long-term absence of fire in the boreal forest (Wardle

Box 1. Definitions of terms.

<i>Succession</i>	Takes place after disturbance to an ecosystem or exposure of new land surface. Succession involves directional changes to both above- and belowground ecosystem components, usually driven by resource availability. Three general stages of succession can be identified; the initial build-up phase, the maximum biomass stage (also referred to as stable state or climax) where nutrient availability matches demand, and finally the retrogressive stage where nutrient limitation leads to a decline in the rate of ecosystem processes.
<i>Soil development</i>	Pedogenesis. The formation and subsequent ageing of soils.
<i>Primary succession</i>	Species replacement on newly exposed substrate with no prior soil development, such as after glacial retreat, volcanic lava flow, land uplift processes, land slides or severe flooding.
<i>Secondary succession</i>	Species replacement after a disturbance to an existing ecosystem that left some organic material and/or plant residues, such as wildfire, logging or hurricanes. The effect of the disturbance varies e.g. depending on the severity of the disturbance. For example, a fire that leaves some soil and plant material has less effect than a fire which consumes all organic matter.
<i>Retrogression</i>	A decline phase characterised by reduced ecosystem productivity and standing biomass. It occurs in the long-term absence of disturbance, in late stages of either primary or secondary succession.
<i>Chronosequence</i>	A series of sites that vary in age since formation or time since last disturbance, and therefore in stage of succession. Variation in other environmental factors across the chronosequence must be minimal to not confound the results. Chronosequences are often used in the study of long-term succession, as they replace time for space.

et al. 2003a), could potentially lead to declining P availability due to increased separation of mineral soil and plant roots. However, the long-term dynamics of P in the boreal forest are not well studied (but see Paper II).

1.4 Effect of herbivory on N and P supply and availability

Herbivory has the capacity to alter plant nutrient supply and growth on a relatively short time scale, particularly for plants that fix N_2 through symbiotic associations with bacteria (Bardgett and Wardle 2003, Bardgett et al. 2005, Frank and Groffman 2009, Ayres et al. 2004). Plant species that fix N_2 are an essential component of early primary succession, where they facilitate other plants and help build up soil N supply by providing N (Walker and del Moral 2003, Bellingham et al. 2005). However, because their N-rich foliage often makes them palatable, N_2 -fixing plants are also a common target for herbivores in early primary succession (Walker and Chapin 1987, Fagan and Bishop 2000). While resilience to herbivory is often weaker in late succession (Walker and Chapin 1987, Crutsinger et al. 2008), the importance of herbivory in altering species interactions and relative performances are usually more important in early to mid successional ecosystems (Augustine and McNaughton 1998). Further, selective grazing herbivores can promote or prevent the advancement of succession (Augustine and McNaughton 1998, Bardgett and Wardle 2003). In nutrient poor forest ecosystems, unpalatable plants have a competitive advantage over palatable plants, which leads to herbivory accelerating successional species replacement and impairing nutrient cycling through promoting plants with low quality litter (Pastor et al. 1988, Davidson 1993, Pastor et al. 1993, Bardgett and Wardle 2003). Meanwhile, in fertile ecosystems such as grasslands, herbivory tends to impair the advancement of succession and promote nutrient cycling and productivity (Archer 1995, Bardgett and Wardle 2003, Sankaran and McNaughton 2004).

Herbivory indirectly affects soil processes linked to soil nutrient availability through affecting plant root C exudation to the rhizosphere (Bokhari 1977, Hamilton and Frank 2001). In fertile ecosystems herbivory often increases C exudation, which stimulates soil microbial activity and has a positive effect on nitrogen mineralisation and plant N uptake (Hamilton et al. 2008). Meanwhile in infertile systems such as many forest and tundra ecosystems, herbivory negatively effects C exudation and consequently N mineralisation (Pastor et al. 1993, Harrison and Bardgett 2004, Sørensen et al. 2008) with potential negative effects for plant N acquisition. Further,

herbivory can also affect the relative availability of N and P for plants, and thus alter the N to P stoichiometry of plants, although this has been little explored. However, Carline et al. (2005) found that herbivory reduced N mineralisation in an upland forest, which led to a corresponding decrease in plant P demand and an increase in the N to P ratio. Conversely, Frank (2008) showed that herbivory in a temperate grassland reduced N mineralization, leading to a decrease in N mineralization and reduction in the plant N to P ratio. By altering the nature of plant nutrient limitation, herbivory also has the potential to affect growth and nutrient acquisition also for those plants that grow nearby and interact with those that are, although not consumed by herbivores themselves (e.g., Pastor et al. 1988, Ayres et al. 2007). However, very few studies have considered the consequences of herbivory for the N and P acquisition of both plants that are actually consumed by herbivores, and their non-eaten neighbours (but see Paper IV). There is evidence of herbivory inducing transfer of N from an N₂-fixing legume to coexisting grass species (Ayres et al. 2007, Saj et al. 2008), but little is known of how herbivory of a plant affects the N: P stoichiometry of its neighbours through interactions above and below ground.

1.5 Objectives

The focus of this thesis is on nitrogen and phosphorus availability for soil and plant communities and its importance in driving ecosystem processes and ecological interactions above and below ground during succession. Papers I-III explore N and P supply and availability to plants and microbes during 5 000 years of post-fire ecosystem succession in the Swedish boreal forest, and how it is linked to ecological factors like biological nitrogen fixation, soil microbial activity and plant nutrient limitation that ultimately affects productivity, decomposition and nutrient cycling. Meanwhile, paper IV investigates the effect of herbivory on plant N: P stoichiometry and its effects on ecosystem function through altering plant interactions and soil processes in relation to primary succession in the New Zealand rainforest. More specifically, the main questions investigated in this thesis were:

1. Is biological N fixation by cyanobacteria associated with feather mosses an important ecological process that contributes to the large build-up on N observed to occur in the humus layer during 5 000 years of ecosystem succession after wildfire in the boreal forest? (Paper I)

2. How does the supply and availability of humus P change during 5 000 years of post-fire ecosystem succession in the boreal forest, and what consequences does it have for ecosystem processes? (Paper II)
3. How do the relative concentrations of N and P in both plant and microbial tissues change during 5 000 years of post-fire ecosystem succession in the boreal forest, and does it reflect the relative availability of N and P in the humus layer? (Paper II and III)
4. How does plant defoliation, such as occurs through herbivory, affect N and P acquisition for both plants that are defoliated and their non-defoliated neighbours and what are the consequences of this for plant interactions during early primary succession? (Paper IV)

2 Material and methods

2.1 Study systems

The chronosequence used in papers I-III is a secondary succession caused by wildfire. It is situated in the northern boreal forest of Sweden (65°55'N to 66°09'N; 17°43'E to 17°55'E), near Arjeplog, where mean annual precipitation is 750 mm and the mean temperature for the warmest and coldest months are +13 °C (July) and -14 °C (January) respectively. The study sites are situated on islands on the two adjacent lakes Uddjaure and Hornavan. Wildfire induced by lightning strike is the main agent of disturbance in the area, and dating of charcoal and fire scars show a strong positive relationship between island area and fire frequency because larger islands are struck more frequently by lightning and therefore burn more often (Wardle et al. 1997). Thirty islands were chosen for study, i.e. ten in each of three size classes: large (>1.0 ha), medium (0.1-1.0 ha) and small (<0.1 ha), with a mean time since last major fire of 585, 2180 and 3250 years respectively (Wardle et al. 2003a). Each island size class represents a different stage of vegetation succession, with *Pinus sylvestris* and *Vaccinium myrtillus* dominating on large islands, *Betula pubescens* and *Vaccinium vitis-idaea* on medium islands and *Picea abies* and *Empetrum hermaphroditum* on small islands (Wardle et al. 2003a) (Fig. 2). The feather mosses *Pleurozium schreberi* and *Hylocomium splendens* dominate the ground layer on all islands. It has previously been shown that these islands collectively form a retrogressive chronosequence with retrogression increasing with decreasing island size as shown by declining plant productivity, nutrient availability and rate of soil processes (Wardle et al. 2003, 2004).



Figure 2. The Swedish island chronosequence. Images from typical large (left), medium (middle) and small (right) islands. Photo: Anna Lagerström.

The study in Paper IV is based on a greenhouse experiment, and designed to answer questions about ecological feedbacks between plants and soil induced by herbivory during early-successional ecosystem development in the Kokatahi Valley of Westland, New Zealand (42° 57' S, 171° 14' E; 400 m a.s.l.). The Kokatahi Valley provides is frequently subjected to disturbances such as flooding, earthquakes and landslides, which create fresh surfaces for plant colonization (Reif and Allen 1988, Bellingham et al. 2001; Fig. 3). The valley is dominated by cool-temperate montane rain forest consisting mainly of evergreen angiosperms (Reif and Allen 1988). The study focused on biotic interactions between plant species that colonize floodplains and landslides. The plants selected for study were the palatable N-fixing shrub *Carmichaelia odorata*, the highly palatable short tree *Fuchsia excorticata* and the moderately palatable evergreen tree *Weinmannia racemosa* (hereafter referred to by genus). Though *Carmichaelia* and *Weinmannia* occur commonly in the Kokatahi Valley, adult *Fuchsia* trees are absent and its highly palatable seedlings are readily removed by invasive Australian brush-tailed possums (*Trichosurus vulpecula*) (Rose et al. 1993, Pekelharing et al. 1998). However, it is likely that adult *Fuchsia* trees were locally common before possums invaded the Kokatahi Valley from the 1880s, and caused *Fuchsia* to disappear (Rose et al. 1993, Pekelharing et al. 1998, Bellingham and Lee 2006). In this light, established *Fuchsia* seedlings planted inside

possum exclosures in the Kokatahi Valley generally thrive (Bellingham and Wardle, unpublished data).

2.2 Experimental design and sampling

Papers I-III were based on field studies on the Arjeplog island chronosequence, where individual islands constitute independent replicate ecosystems (Wardle 2002), with ten islands in each of the three groups small, medium and large islands. The islands together function as a ‘natural experiment’ (Fukami and Wardle 2005), in which variation in island size determines wildfire history, but across which all other extrinsic driving factors remain relatively constant. This range of fire histories in turn causes a gradient of various ecosystem properties and process rates, including productivity, microbial activity, concentration of humus N, phenolic compounds, litter quality and plant species succession (Wardle et al. 1997, 2003). The work reported in Papers I-III is based on sampling and measurements across this natural experiment. Sampling on each island took place in a 20 x 20 m study area, placed at equal distance from the shore for all island size classes, to minimize differences in edge effect (Wardle et al. 2003).



Figure 3. Primary successional surfaces in the Kohkatai Valley, Westland, New Zealand. *Carmichaelia odorata* growing on a river bank (left) and fieldwork on a recent landslide (right). Photo: Anna Lagerström.

In Paper I, moss shoots for measurement of N_2 fixation were collected by hand from each island on three occasions, i.e., early, mid and late in the growing season (since N_2 fixation is known to fluctuate with season; DeLuca et al. 2002), over each of two years. At each sampling occasion, 30 shoots of *P. schreberi* and 10 shoots of *H. splendens* (which has larger shoots than *P. schreberi*) were sampled from each island. In Paper II, soil for chemical and microbial analysis was collected as three cores (10 cm diameter; at least 2 m between coring sites) from each island, and they were later bulked into one composite sample for each island. Only the top 10 cm of the humus was sampled, partly because that is where the main part of microbial activity occurs (Chapin et al. 1978) and partly because that was the maximum humus depth on most large islands. In Paper III, fresh foliage and litter for nutrient concentration analysis was collected from the six vascular plant species that dominate the entire sequence. Ten trees of each of the three main tree species (when present), and of each of the three main dwarf shrub species were sampled once per growing season on each island for each of two years. For each plant species, all green leaves were bulked within each island to yield a single composite sample, as was all leaf litter.

In Paper IV a controlled pot experiment was set up in a glasshouse to study the combined effect of (simulated) herbivory and competition on plant growth, nutrient status, competitive interactions and soil microbial activity for the three plant species. Standardised nutrient poor soil was used for all treatments. The experiment consisted of 18 treatments in total. Six of these were monocultures of the three species, each either clipped or unclipped (two seedlings per pot), with clipping serving to simulate defoliation caused by herbivores (Mikola et al. 2001, Ayres et al. 2007, Hamilton et al. 2008). The remaining 12 treatments each contained two species mixtures (two seedlings of each species per pot), involving four treatments for each of the three two-way combinations of the three species. The four treatments were no species clipped; only one of the species clipped, only the other of the species clipped, and both species clipped. As such, the experiment was set up as an additive competition experiment (Wilson 1988, Snaydon 1991) combined with clipping treatments, and has the advantage of allowing direct examination of the effects of each species on each other species (Snaydon 1991, Dehlin et al. 2004). The experiment was set up in a randomized block design, with six replicate blocks.

2.3 Methodological aspects

2.3.1 Nitrogen fixation measured with acetylene reduction

Nitrogen fixation rates, investigated in Paper I, were analysed using the acetylene reduction method (Schöllhorn and Burris 1967) as described by Zackrisson et al. (2004). Analysis was always started within 24 hours of sampling, to avoid problems with storage of the mosses. This method is an indirect measure of N_2 fixation in that it measures the reduction of acetylene gas to ethylene, which is proportional to reduction of N_2 gas during N_2 fixation. To convert values of rates of ethylene production to rates of N_2 fixation in *P. schreberi*, we used a previously determined conversion factor for this species of 1 mole of N_2 reduced to 3 moles of acetylene reduced (DeLuca et al. 2002). The conversion factor between acetylene reduction and N_2 reduction had not been determined for *H. splendens* prior to this study, but moss sample incubation with acetylene and with $^{15}N_2$ gas as described by DeLuca et al. (2002), showed that the ratio of 3 moles of acetylene to 1 mole of N_2 gas is also the correct conversion factor for *H. splendens*. It has been suggested that measuring N_2 fixation rates with acetylene reduction may lead to an underestimation of N_2 fixation as nitrogenase activity is known to decline in the presence of acetylene (Minchin et al. 1983). However, the primary aim was to compare relative fixation between island size classes, and there is no reason to believe that any reduction of this type would lead to confounding effects across size classes.

2.3.2 Microbial respiration measurements

In paper II, the relative limitation of the microbial community by N and P was investigated by using the approach of (Nordgren 1988, 1992), which involves adding these nutrients to soil subsamples in pre-determined amounts and then measuring soil respiratory kinetics over time. Soil microbial respiration was measured using a respirometer (RespiCond IV; A. Nordgren Innovations, Djäkneboda, Sweden), which calculates the amount of respired CO_2 from the change in conductivity caused by accumulated CO_2 in a KOH trap, as described by Nordgren (1988; 1992). The respirometer enables detailed measurements of respiration kinetics including basal respiration, substrate-induced respiration, lag phase, time taken for microorganisms to start exponential growth (after substrate addition), exponential growth rate, maximum respiration and time to maximum

respiration (Nordgren 1988, Giesler et al. 2004). In combination, these measurements make up a comprehensive picture of the relative availability of the added nutrients to soil microorganisms over time. Though the observed results do not represent natural conditions, they give a good estimation of potential microbial nutrient limitation (Nordgren 1988, 1992, Vesterdal 1998, Giesler et al. 2004).

In paper IV, microbial basal respiration (BR) and substrate-induced respiration (SIR) was measured using an infrared gas analyser. They are used as relative measures of microbial activity and glucose-responsive microbial biomass respectively (Anderson and Domsch 1978, as described by Wardle 1993).

2.3.3 Sequential extraction of phosphorus

The Hedley fractionation procedure was used in Paper II to study soil P forms and how they are affected by biological and geochemical processes across the island chronosequence. Sequential extraction of P was performed in five steps using the approach of Hedley and Stewart (1982) as modified by Binkley et al. (2000) and Giesler et al. (2004). The term “labile P” is used throughout Paper IV and this thesis to refer to P fractions extracted by anion exchange membranes and NaHCO_3 , which are assumed to be labile and readily available to plants and microbes (Cross and Schlesinger 1995). Other fractions are NaOH-extractable P, which is assumed to contain P that is adsorbed to Al and Fe complexes, and organic P that is more resistant compared to the labile fractions but still available over an intermediate time scale (Cross and Schlesinger 1995). The HCl-fraction consists of occluded inorganic P and the residual fraction of occluded inorganic P and stable organic P. Stable organic P has traditionally been considered unavailable to plants and microbes (Cross and Schlesinger 1995), but recent evidence suggests that there are various mechanisms through which plants can access stable organic P (Turner 2008).

2.3.4 Plant traits and weighted averages

For Paper III leaf and litter traits were measured for each vascular plant species on each island. These included Specific Leaf Area (SLA), % leaf N and P, % litter N and P, leaf and litter N: P ratio, and N and P resorption. Specific leaf area determined as a measure of the ratio of leaf area to leaf dry

mass (Garnier et al. 2001). Resorption (Killingbeck 1986) was calculated as the percent reduction in N or P of litter compared to fresh leaves (Aerts 1996).

In order to study leaf traits at a community-level across the island gradient, weighted means of leaf traits were used as according to Garnier et al. (2007). Calculation of weighted mean traits makes it possible to quantify community-level values for any given leaf trait based on the trait value for each species and its relative contribution to total community biomass or productivity. For each leaf trait measured, this approach was used to calculate a community-level value of that trait for each island, both using live biomass for each vascular plant species (data in Wardle et al. 1997) and productivity for each plant species (data in Wardle et al. 2003) for each island. The following equation according to Garnier et al. (2007) was used:

$$trait_{agg} = \sum p_i \times trait_i$$

where $trait_{agg}$ is the aggregated trait (or community-level) value for each island, p_i is the relative contribution of species i to the community (in this study expressed as biomass or as productivity), and $trait_i$ is the value of the leaf trait in species i . Weighted mean traits were calculated at both the whole community level and within the main functional groups (trees, and dwarf shrubs).

2.4 Statistical evaluation

In papers I-III, individual islands were used as the basis of replication for all analyses. The effect of island size class on measured variables (e.g. N_2 fixation, respiration parameters, soil P forms, and foliar nutrients) was tested using Analysis of Variance (ANOVA), followed by Tukey's post-hoc test at $P \cdot 0.05$. In paper II, the relationships between microbial response variables and soil chemical data were analysed using multiple stepwise regression, into which variables were entered when probability of F was less or equal to 0.05. As more variables are entered stepwise into the regression the probability of F for a previously entered variable can change, and when this happened variables were removed if their influence on the dependent variable had a probability of F higher than or equal to 0.10. The relationships between measured variables and actual islands size and time since fire were analysed with linear regression (i.e., humus N, C: N and N: P ratios in paper II, and humus N and N fixation in Paper I). In Paper IV, indices of 'competition intensity' and 'competitive balance' presented by

Wilson (1988) were used to determine how competitive interactions between species were affected by clipping. ANOVA was used to test for the effects of clipping and neighbour on plant growth, N and P content and microbial activity.

3 Results and Discussion

3.1 Input, supply and availability of N

The results from Paper I showed a marked increase in N_2 fixation rate in both of the two dominant feather moss species (*Hylocomium splendens* and *Pleurozium schreberi*) with decreasing island size, i.e. during the retrogressive stage of succession. Input of N through N_2 fixation was comparable to atmospheric N deposition on small islands, while on large and medium islands input through atmospheric deposition exceeded that added through N_2 fixation. This larger total input of N on small islands helps explain the increased humus N amounts and N: P ratios that occurs on small islands (Wardle et al. 1997, 2004a) These results add to earlier work in this system that has shown significant sequestration of humus C and N in the long-term absence of fire to also occur through the impairment of decomposition and microbial activity, and greater recalcitrance of plant litter, during retrogression (Wardle et al. 2003b, Dearden et al. 2006, Jonsson and Wardle 2008, Wardle et al. 2009). The higher moss abundance probably also contributes to the sequestration of N in the humus of small islands through producing litter that decomposes very slowly compared to that from vascular plants (Oechel and Van Cleve 1986, Lang et al. 2009). The fact that N_2 fixation was significantly related to island area but not to time since fire (Paper 1) is probably due to that N_2 fixation is strongly linked to succession after fire (Zackrisson et al. 2004). While island size reflects the cumulated fire frequency of an island since its creation, charcoal dating only provides time since the most recent fire but we do not know if that fire was severe enough to reset succession.

The increasing N_2 fixation rate in the long-term absence of fire in the boreal forest occurs in combination with a previously documented decrease in N availability (Wardle et al. 1997, De Luca et al. 2007) caused by N immobilisation by phenolic substances, which occur in higher concentrations on small islands (Wardle et al. 1997, Nilsson and Wardle 2005). There are several reasons as to why N_2 fixation may increase as island size decreases or as retrogression proceeds. First, Higher availability of N in early succession can lead to a higher deposition of N on moss carpets from canopy throughfall, which in turn impairs N_2 fixation in feather mosses (DeLuca et al. 2008). As retrogression proceeds on the small islands, there is less standing tree biomass (Wardle et al. 2003a) and so canopy throughfall will be less and impairment of N_2 fixation by throughfall will be reduced. Second, moisture is an important factor regulating N_2 fixation (Chapin et al. 1990, Turetsky 2003), and the deeper humus layers that form during retrogression in the studied chronosequence (Wardle et al. 2003a) as well as the thicker moss layer, most likely enhances moisture conservation which should benefit N fixation on small islands (cf. Oechel and van Cleve 1986). Third, recent evidence suggests a positive connection between N_2 fixation in feather mosses and the roots of the nutrient-conserving tree *Picea abies*, which is dominant on small islands (Gundale et al. In press). Finally, moss growth and associated N_2 fixation could be inhibited by competition from surrounding plants, and previous work shows a lower intensity of plant competition on the small islands (Wardle and Zackrisson 2005, Wardle et al. 2008a).

The fact that the studied area has low atmospheric N deposition (around $2 \text{ kg ha}^{-1} \text{ yr}^{-1}$), and that cyanobacteria associated with feather mosses are the only widespread N_2 -fixing organisms in the boreal forest (with the exception of *Alnus* species that are absent and some lichen species that are scarce in the study system) means that N input from N_2 fixation becomes an increasingly important N source as retrogression proceeds. On the small islands N_2 fixation by cyanobacteria is comparable to atmospheric deposition as an ecosystem N source, and is responsible for increasing total annual ecosystem N inputs by 40 % relative to large islands. The availability of N fixed in feather mosses to plants and soil microbes is unclear. Nitrogen added through atmospheric deposition is usually in mineral or other relatively simple forms, while feather mosses have a very efficient N conservation strategy in which they recycle N from old to new segments (Eckstein 2000), and produce very slowly decomposing litter (Oechel and Van Cleve 1986, Lang et al. 2009). Further, mosses can be highly competitive at acquiring and retaining N (Ayres et al. 2006), and can compete very effectively with

plants for N in snow cover during snowmelt (Forsum et al. 2008). This adds to results from earlier studies, which have shown N to be less available on small islands due to immobilisation of N by phenolic compounds (Wardle et al. 1997, Nilsson and Wardle 2005).

3.2 Supply and availability of P

The original source of P is weathering of the bedrock and mineral soil, but after tens to hundreds of thousand years of weathering, leaching and chemical immobilisation, the source and availability of P diminishes greatly (Walker and Syers 1976, Crews et al. 1995, Turner 2007). The time scale at which this occurs is dependent on parent material, climate, especially precipitation (Porder and Chadwick 2009), and external P sources (Chadwick et al. 1999). The age of studied sites in Hawaii, New Zealand and Australia where P depletion causes P-limitation range from 22 000 to 4.1 million years, while the Swedish island chronosequence studied in this thesis only spans 5 000 years of ecosystem development. However, Hoffland et al. (2002) showed that weathering by mycorrhizal fungi could significantly deplete mineral P in as little as 2 000 years in a boreal forest chronosequence. In addition, accumulation of very deep organic humus layers in the long-term absence of fire in the boreal forest could hamper plant access to mineral P by separating mineral soil and plants. However, in northern ecosystems, where low temperature and plants traits contribute to slow decomposition rate (compared to tropical and temperate areas), biological cycling of P is more likely to be an important driver of P availability (Chapin et al. 1978, Wood et al. 1984).

The results from the Hedley fractionation of P in Paper II showed that total P and the largest P fractions did not decline during retrogression or change significantly across the chronosequence. This is contrary to previous studies on P fractions in retrogressive chronosequences (Walker and Syers 1976, Walker 1983, Chapin et al. 1994, Crews et al. 1995, Vitousek 2004) and suggests that leaching and geochemical immobilisation of P play a minor roll in promoting ecosystem retrogression during 5 000 years of succession on these islands. The lack of large changes between major P fractions in the top 10 cm of the humus as revealed in this study is unexpected since P availability tends to decline during soil development, both during the process of podsol formation (which increases the geochemical sinks for P; Starr 1991) and through depletion of primary mineral P by weathering (Hoffland et al. 2000). Substantial accumulation of P in organic P pools may

account for the lack of loss through geochemical processes. Total organic P accounted for 73 ± 0.01 % and 74 ± 0.01 % of the total P on large and medium islands respectively, and increased to 78 ± 0.01 % on small islands ($F = 6.25$, $P = 0.006$, one-way ANOVA). The increase in the large organic P pool on small islands can be linked to a reduced rate of decomposition of organic material on small islands (Wardle et al. 2003b, 2004a, Jonsson and Wardle 2008). Together, these results suggest that slow biological P cycling is the main control of P supply across this boreal forest chronosequence.

In contrast to organic P, the smaller fraction of labile (inorganic) P declined from medium to small islands (Paper II). This result is consistent with earlier studies that have demonstrated declining P availability in the retrogressive stage of succession (Walker and Syers 1976, Crews et al. 1995, Wardle et al. 2004a, Coomes et al. 2005, Vitousek 2004, Selmants and Hart 2008). However, given the strong biological control of P cycling in this boreal forest, it is unlikely that the decline in labile P on small islands was caused by geochemical factors as has been shown for other retrogressive chronosequences (Walker and Syers 1976, Crews et al. 1995, Vitousek et al. 2004, Selmants and Hart 2008). The P fractions that bind to Al and Fe (the NaOH-extractable P_i fraction) had low concentrations throughout the chronosequence (Paper II), which implies that immobilization of P through sorption to Al and Fe is probably not connected to the decrease observed in labile P (Giesler et al. 2004). The release of P in boreal humus soils is primarily driven by enzymatic degradation of organic P (Attiwill and Adams 1993), which is likely to be inhibited by the lower litter quality and decomposition rates on the small islands (Wardle et al. 2003b, 2004a), probably leading to lower quality of organic P on small islands. Thus, the quality of organic C, which is frequently the main limiting element for soil microbes (Ekblad and Nordgren 2002), probably determines P mineralisation rate and availability in the studied system.

3.3 Microbial nutrient limitation and N: P stoichiometry

Studies of nutrient limitation of the soil microbial biomass (in addition to plant nutrient limitation) can give a more complete picture of nutrient limitation at the ecosystem level (Cleveland and Liptzin 2007). Cleveland and Liptzin (2007) found a relatively consistent critical ratio of N: P for soil microbial biomass globally, above which the microbes are P limited and below which they are N limited (Table 1). In their study, the critical ratio

varied somewhat among vegetation types and subsets of the microbial biomass due to variation in environmental nutrient availability and microbial requirements. The results in paper II showed that though the microbial biomass N: P ratio across all island size classes was below the critical ratio identified by Cleveland and Liptzin (2007), and was therefore indicative of N rather than P limitation, the microbial N: P ratio was lower on medium islands than on large and small islands. This was due to higher microbial P levels on medium islands, and to microbial N not varying between island size classes (Paper II). This could be a result of higher quality of litter inputs on the medium islands resulting from domination by deciduous species (notably *B. pubescens*) that may promote microbial nutrient access. These results show that on these islands, soil microbial biomass does not reflect variation across the gradient in previously determined humus mineral N concentration (Wardle et al. 1997), but it does reflect the decline in labile P on retrogressive small islands. These results suggest that microbes are either not dependent on mineral N or are superior competitors for N compared with plants (which do vary in N content across the island size classes). It also supports the previous conclusion that because C is often the main limiting element for microbes (Ekblad and Nordgren 2002), the rate of degradation of organic P is dependent on organic C quality.

The finding that the microbial biomass N: P ratio was within the range indicative of N limitation (see Table 1) across all island size classes was confirmed by measures of microbial respiration after addition of C and N or P. Specifically, all soils gave a much stronger respiratory response to addition of C+N than to C+P across all island size classes. We did not find the relative respiratory response to P to be stronger on small than on large islands, as would have been expected if P was increasingly limiting relative to N during retrogression. However, the concentration of labile P was the best predictor of a range of microbial respiration parameters, including basal respiration (Paper II). Though microbial respiration is a good measure of relative microbial activity and glucose-responsive biomass (Anderson and Domsch 1978), different microbial processes can respond differently to variation in resources. Therefore it is not certain that the decline in labile P does not affect any microbially driven soil process. The significant correlation between labile P and microbial respiration parameters implies that P has some influence on soil microbial activity, despite N being the main limiting nutrient for growth. This is in agreement with the concept of multiple resource limitation of microbially driven ecosystem processes (Kaspari et al. 2008).

3.4 Plant nutrient limitation and N: P stoichiometry

In Paper III, plant leaf traits were studied at the level of the whole plant community, functional group (trees versus shrubs) and individual species across the island chronosequence. At the whole community and functional group levels, the weighted traits averages approach (Vile et al. 2006, Garnier et al. 2007, Quested et al. 2007, Fortunel et al. 2009) was used, so that each aggregate plant trait value was determined by the value of that trait of each species and its proportional contribution to either total biomass or total productivity. Weighted trait averages revealed trends across island size classes at the levels of community and functional group that could not be detected at the individual species level. This illustrates the importance of shifting plant species composition driving variation in leaf traits across the island area gradient. It also demonstrates the usefulness of weighted traits averages for detecting trends across environmental gradients for which species composition also shifts (Vile et al. 2006, Garnier et al. 2007, Quested et al. 2007, Fortunel et al. 2009). The results contradicted expectations through showing that leaf N and P at the community level did not decrease along with previously documented trends of declining humus N and P availability with decreasing island size (Wardle et al. 1997, Wardle and Zackrisson 2005; also Paper I). Instead, biomass-weighted leaf N, P and SLA actually increased as island size decreased at the community level. The change in leaf traits was mainly a result of shifting species composition across the island sequence, and not within species variation, as leaf traits differed much more between than within species. This is inconsistent with Richardson et al. (2004, 2008), who found that leaf N and P decreased within species across a retrogressive chronosequence. No leaf traits at the community level responded to island size classes for the productivity-weighted data, but this is explained by opposing trends in leaf traits of trees and dwarf shrubs cancelling each other out. Because total biomass is dominated by trees, particularly *Pinus*, the biomass-weighted means at the community-level resemble those of the tree species. In contrast, as trees and dwarf shrubs contribute more evenly to total productivity (Nilsson and Wardle 2005), productivity-weighted values at the community level reflect in trends in both groups.

When the weighted traits average approach was applied within functional groups trees and dwarf shrubs showed largely opposite trends in leaf traits (Paper III). Weighted traits values for trees revealed that trees overall generally had lower foliar N, litter N, foliar P, litter P and SLA on larger islands. Further, for trees, biomass-weighted values for both N and P

resorption were higher on large islands. On the contrary, weighted traits values for dwarf shrubs as a group revealed that they generally had higher foliar N, foliar P, litter P and SLA on large islands, with N resorption peaking on medium islands and P resorption being lower on large islands. These contrary trends for both nutrient concentration and resorption between trees and dwarf shrubs points to very different nutrient acquisition strategies between the two functional groups. For example, shrubs may be relying primarily on mineral N and P, which declines with island size (Paper II), while trees appear to have a different strategy, possibly relying on more recalcitrant P (Turner 2008) and N that is fixed biologically in feather mosses (Gundale et al. In press). This may be a result of small islands with their deeper humus layers (Wardle et al. 2003a) and higher N₂ fixation rates (Lagerström et al. 2007) providing an opportunity for resource partitioning between trees and shrubs that does not exist on larger islands, or simply because resource competition over long time scales without disturbance leads to niche divergence (Wilson 1999). The potential for this type or resource partitioning to be greater on small islands is consistent with the lower intensity of competition previously documented on the small islands (Wardle and Zackrisson 2005, Wardle et al. 2008).

At the species level, variation in leaf traits was larger between different species than within species across the island size gradient. For all studied leaf traits, only three or fewer of the six dominant species responded to island size class at the within-species level (Paper III). This is in contrast to Richardson et al. (2004, 2008), who showed that leaf nutrient concentrations declined with soil fertility during retrogression both at the plant community and species level. However, in this study, data for the individual species responses show that some of the variation in the weighted trait average values at the whole community and functional group levels described above can be explained by within-species variation of those species that dominated the community or functional group. For example, SLA was significantly less on small islands for *Betula* and *V. myrtillus* and significantly less on large islands for *Pinus*, but the weighted traits values showed lowest SLA values on large islands because of the dominating influence of *Pinus*. In addition, some traits that were weak at the species level were strengthened by the weighted trait analysis. For example, foliar N was significantly higher on large islands for dwarf shrubs as a group when weighted traits values were used, but for the species data both *V. myrtillus* and *V. vitis-idaea* had lower foliar N on large islands. These results indicate that variation in community-level leaf traits can be more strongly influenced by shifts in the composition of species or functional groups than by

environmental factors (Cornelissen et al. 1997, Dorrepaal et al. 2005) such as changes in soil nutrient availability during retrogression.

The ratio of leaf N to P can be a useful indicator of the nature of plant nutrient limitation (Koerselman and Meuleman 1996, Tessier and Raynal 2003, Güsewell 2004, MacGroddy et al. 2004). At the community level and for the trees, the ratio of N: P was not affected by island size class when weighted traits averages were used. However, dwarf shrub N: P ratio was higher on small islands when weighted averages were used, consistent with the soil N: P ratio in this study system (Wardle et al. 2004a), and with other studies of leaf N: P ratios during retrogression (Vitousek et al. 1995, Richardson et al. 2004, Wardle et al. 2004a, Coomes et al. 2005). At the species level, the ratio of N: P was higher on small than on large islands for *V. vitis-idaea* foliar and *V. myrtillus* litter, and for *Picea* litter N: P ratio was higher on small than on medium islands. However, these increased N: P ratios on small islands do not indicate a shift in nutrient limitation, as they are all considerably below the critical N: P ratio for plants (Koerselman and Meuleman 1996, Tessier and Raynal 2003, Güsewell 2004, MacGroddy et al. 2004). The foliar and litter N: P ratios of individual species ranged from around five for the evergreen *Picea*, to around nine for the deciduous *Betula* and *V. myrtillus*. These values are much lower than has been observed in other retrogressive chronosequences (Vitousek et al. 1995, Richardson et al. 2004, Wardle et al. 2004a, Coomes et al. 2005), and are probably due to the importance of biological nutrient cycling driving nutrient availability in the studied system, as opposed to loss and immobilisation of P through geochemical processes.

Nutrient resorption prior to leaf abscission is an important nutrient conservation strategy for many plant species (Aerts 1996, Killinbeck 1996). The results from Paper III showed that resorption efficiency was negatively linked to leaf N and P concentration, which is consistent with global trends (Kobe et al. 2005). Previous studies have found nutrient resorption efficiency to respond either positively (Herbert and Fownes 1999, Cordell et al. 2001, Richardson et al. 2005, Castle and Neff 2009) or neutrally (Chapin and Kedrowski 1983, Aerts 1996) to declining soil fertility. In contrast, we found that while weighted trait average values for N and P resorption for the dwarf shrubs did vary with island size, resorption for the trees and for the whole plant community actually decreased as islands became smaller and nutrients therefore became more limiting. As such, nutrient resorption does not serve as a good indicator of the severity of plant nutrient limitation in this study system. Resorption proficiency (% nutrient content in senesced litter; Killinbeck 1996) has been suggested to better reflect plant adaptation

to infertile environments because it reflects the level to which the plant is able to resorb nutrients, rather than the proportions of nutrients in the fresh leaves (which can themselves vary seasonally) that are resorbed (Killingbeck 1996). In Paper III, *V. vitis-idaea* had the lowest resorption proficiency (i.e. litter N and P %) of all species, though *Pinus* also had low litter P. The disproportionally large contribution of *Pinus* and *V. vitis-idaea* to total productivity compared to the other species (Nilsson and Wardle 2005) suggests that low resorption proficiency may be associated with having competitive advantages in this study system.

3.5 Effect of herbivory on the nutrient status and interactions of plants

Plants that are associated with N₂ fixing bacteria play an important role in building up N supply and thereby facilitating the growth of other species early in primary succession (Walker and del Moral 2003, Bellingham et al. 2001, 2005). The results from Paper IV show that simulated herbivory of a woody N₂-fixing shrub (*Carmichaelia*) negatively affected its growth and N-fixation, and it also removed the positive effect that *Carmichaelia* had on leaf N of the neighbour tree *Weinmannia* when not clipped to simulate herbivory. The negative effect of simulated herbivory of *Carmichaelia* on growth and nodulation, as well as on growth and N uptake by a neighbouring plant, is opposite to the findings of some earlier studies on the effect of herbivory on N release from the plant (Hamilton and Frank 2001, Ayres et al. 2007, Hamilton et al. 2008, Saj et al. 2008). However, those studies were carried out on grasses, and in grasslands herbivory generally have a more positive effect on nutrient cycling than for ecosystems dominated by woody plant species (Pastor et al. 1993, Stark et al. 2000, Harrison and Bardgett 2004, Sørensen 2008). My results show that herbivory of a woody N₂-fixing shrub has the potential to impair build-up of N supply and N supply to non-N₂ fixing species during early primary succession, potentially impairing ecosystem nutrient cycling and productivity.

The three studied plant species in Paper IV differed considerably in the responses of their leaf N and P to herbivory, which ranged from a strong negative response in *Carmichaelia* to an almost complete lack of response in the palatable tree *Fuchsia*. The presence of *Carmichaelia* caused higher N concentrations in *Weinmannia* leaves and higher microbial activity in soils of

both *Weinmannia* and *Fuchsia*, but that effect disappeared when *Carmichaelia* was clipped. However, even when it was clipped, *Carmichaelia* caused a lower leaf P and higher leaf N: P ratio in *Weinmannia*, compared to when *Weinmannia* had no neighbour or *Fuchsia* as the neighbour (Paper IV). The N: P ratio of *Weinmannia* leaves increased from about three when grown without a neighbour or with *Fuchsia* as the neighbour to six when grown with clipped *Carmichaelia* and 12 with unclipped *Carmichaelia*. These results show that the presence of unclipped *Carmichaelia* had raised the N: P ratio of *Weinmannia* towards the level at which P can become relatively more limiting than N (Güsewell 2004). The increased N: P ratio was inevitably a result of higher relative N availability (Carline et al. 2005, Frank 2008). Previous studies have showed that herbivory can induce P limitation in grassland (Frank 2008) and N limitation in regenerating woodland (Carline et al. 2005), but this study has demonstrated that the effect of defoliation on the N: P ratio of *Weinmannia* can be insignificant when compared to the effect of defoliation of its N₂ fixing neighbours.

Plant species response to herbivory in terms of growth, survivorship and competitive ability is a major driver of plant community composition (Grime 1979, Augustine and McNaughton 1998, Grime 2001). Calculation of indices revealed that herbivory had a significant negative effect on the competitive ability of *Carmichaelia*, but less so for *Weinmannia* and *Fuchsia*. Further, although *Carmichaelia* induced higher leaf N in *Weinmannia* when they were grown together, this did not favour the growth of *Weinmannia* (Paper IV). Instead the presence of unclipped *Carmichaelia* caused significantly reduced shoot and root biomass in both *Weinmannia* and *Fuchsia*, although this effect disappeared when *Carmichaelia* was clipped. Earlier studies have suggested that *Carmichaelia* has positive effects on neighbouring plants (Bellingham et al. 2001); this study shows despite that *Carmichaelia*'s positive effect on a neighbour's leaf N and soil microbial activity, it suppresses the growth of its neighbours through competition at least when not defoliated (Paper IV). The balance between facilitation and competition is important for successional development in early primary succession (Walker and Chapin 1987), and these results show that this balance can be drastically altered by which species are defoliated. Further, growth, competitive interactions, and associated soil microbial activity for both *Weinmannia* and *Fuchsia* were more responsive to defoliation of neighboring *Carmichaelia* than to defoliation of themselves. This highlights that herbivory of a neighbouring plant can have more effect on plant growth, soil processes and plant interactions than does herbivory of the focal plant.

3.6 Conclusions

A summary of the findings provided in this thesis about retrogressive ecosystem succession on the Arjeplog island chronosequence, together with findings of earlier studies in this system are given in Table 2. The results of this thesis have showed that unlike other well studied retrogressive chronosequences (Walker et al. 1983, Richardson et al. 2004, Vitousek 2004, Wardle et al. 2004a Coomes et al. 2005, Selmants and Hart 2008), retrogression is not linked to a shift from N to P limitation in this chronosequence. Instead, N remains the main limiting element during retrogression, and the increasing soil N: P ratio is caused by accumulation of N in the humus, driven by increased N_2 fixation in the retrogressive stage. Since N limitation is apparent on the small islands despite high N_2 fixation, it appears that much of the N input to those islands is locked up in unavailable forms, for example through complexing with polyphenolics, that are not readily accessible to vascular plants or microbes. These results also point to the importance of N_2 fixation as a N source in late successional systems, despite the widely held view that biological N_2 fixation is usually only high in early successional ecosystems. Further, the results of this thesis show that biological processes linked to plant species effects (Wardle et al. 1997, 2003a), and organic C, quality are more important in regulating P availability in this chronosequence than are factors such as weathering and P occlusion, which have been shown to be of importance in other chronosequences (Walker and Syers 1976, Crews et al. 1995, Hoffland 2002, Turner et al. 2007). In other words, for the studied chronosequence there is no evidence for strong limitation of available P forms developing as retrogression proceeds.

The lack of a clear connection between the declining soil mineral N or P availability and plant N or P characteristics during retrogression indicates that plants have access to recalcitrant pools of nutrients such as organic N (Näsholm et al. 2009) and forms of P that are more strongly bound (Turner 2008). Interestingly, nutrient acquisition strategies appear to differ

Table 2. Characteristics of the Swedish island chronosequence. Underlined information is from studies in this thesis.

	Large islands	Medium islands	Small islands
	Successional development		
Dominating plant species	<i>Pinus sylvestris</i> <i>Vaccinium myrtillus</i>	<i>Betula pubescens</i> <i>Vaccinium vitis-idaea</i>	<i>Picea abies</i> ¹ <i>Empetrum hermaphroditum</i>
Plant characteristics	High allocation of C to growth Low plant diversity High competition intensity Low seedling establishment		High allocation of C to phenolics ¹ Higher plant diversity ² Low competition intensity ^{3,4} High seedling establishment ⁴
Productivity	Higher standing biomass Higher litter fall		Lower standing biomass ⁵ Lower litter fall ⁵
Litter	Low phenolics Low % twigs		High phenolics ¹ High % twigs ⁶
Decomposition	Generally faster		Generally slower ^{6,7,8}
Soil characteristics	Low C sequestration <u>Low total N</u> High mineral N <u>Total P unchanged across all size</u> <u>Labile P peaks on medium islands</u> ¹¹ <u>Low N: P ratio</u>		High C sequestration ^{5,9} <u>High total N</u> ^{1,10} Low mineral N ¹ <u>classes</u> ¹¹ <u>High N: P ratio</u> ^{9,10}
Leaf traits	<u>Trees low N and P %</u> <u>Shrubs high N and P %</u> <u>Trees higher N and P resorption</u>		<u>Trees higher N and P %</u> ¹² <u>Shrubs lower N and P %</u> ¹² <u>Shrubs higher N and P resorption</u> ¹²
N ₂ fixation	<u>Low</u> No significant link to tree roots		<u>High</u> ¹⁰ Positive link to tree roots ¹³
Invertebrates	Lower invertebrate density Lower species richness Less aquatic insects on vegetation		Higher invertebrate density ¹⁴ Higher species richness ¹⁴ More aquatic insects on vegetation ¹⁵
Herbivory	Low insect herbivory $\delta^{15}\text{N}$ low		High insect herbivory ¹⁶ $\delta^{15}\text{N}$ high ¹⁷

¹Wardle et al. 1997, ²Wardle et al. 2008b, ³Wardle and Zackrisson 2005, ⁴Wardle et al. 2008c, ⁵Wardle et al. 2003, ⁶Dearden et al. 2007, ⁷Wardle et al. 2004, ⁸Jonsson and Wardle 2008, ⁹Jonsson and Wardle 2009a, ¹⁰Paper I, ¹¹Paper II, ¹²Paper III, ¹³Gundale et al. In press, ¹⁴Jonsson et al. 2009, ¹⁵Jonsson and Wardle 2009b, ¹⁶Crutsinger et al. 2008, ¹⁷Hyodo and Wardle 2009.

depending on plant functional group (trees versus shrubs) and stage of succession, with increasing divergence between functional groups during retrogression. This is consistent with a greater intensity of competition in early succession (Nilsson and Wardle 2005, Wardle et al. 2008a) and greater resource partitioning during late succession (Wilson 1999) and as retrogression proceeds. Importantly, changes across the chronosequence in community-weighted values in those plant traits that are related to nutrient acquisition mainly resulted from shifting species composition, because traits varied more between than within species. It is concluded that aboveground responses to retrogressive development are dependent on plant adaptations, specific species characteristics, and shifts in species composition, which makes them hard to predict.

In addition to considering long term trends in aboveground and belowground processes and feedbacks that lead to ecosystem retrogression over thousands of years, this thesis also considered related issues over a shorter time scale, i.e., during the early stage of vegetation succession, i.e. over decades, development that occurs over the order of decades, with focus on a primary successional system in New Zealand. While the Swedish island system was primarily subjected to disturbance by wildfire that affected aboveground and belowground processes, the principal factor of disturbance in the New Zealand system was herbivory by introduced invasive mammals. Accordingly, this thesis has also demonstrated that herbivory can have important effects on how N_2 fixing plants influence other surrounding plant species and soil properties during early succession. Furthermore, the results from Paper IV showed that the N: P stoichiometry of one important non- N_2 fixing plant species was more responsive to the simulated herbivory of a neighbouring N_2 fixing plant than of itself. This shows the capacity of herbivory to alter nutrient status and competitive interactions not only of the targeted plant but also its neighbours, which have potential to ultimately affect species composition, nutrient cycling and productivity as succession proceeds.

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