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Relationships between tree and soil properties in *Picea abies* and *Pinus sylvestris* forests in Sweden

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Abstract

The exchange of elements between plants and the soil in which they are growing creates reciprocal control of their element composition. Within plants, the growth rate hypothesis from ecological stoichiometry implies a strong coupling between C, N, and P. No similar theory exists for predicting relationships between elements in the soil or relationships between plants and the soil. We used a dataset of element concentrations in needles and humus of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) forests in Sweden to investigate the extent to which relationships between elements (C, N, P, S, K, Ca, Mg, Fe, Mn, Al) can be observed within and between plants and soils.

We found element composition to be more strongly controlled in needles than in humus. Elements that are covalently bound were also more strongly controlled, with no apparent differences between macronutrients and micronutrients. With the exception of N/C, there were surprisingly few relationships between elements in needles and humus. We found no major differences between the two tree species studied, but investigations of additional forest types are needed for firm conclusions. More control over element composition was exercised with respect to N than C, particularly in needles, so it might be advantageous to express nutrient concentrations relative to N rather than on a dry weight or carbon basis. Variations in many ecosystem variables appeared to lack ecological significance and thus an important task is to identify the meaningful predictors.

Key words: conifers, ecosystem variables, element composition control, stoichiometry, humus, needles.

Introduction

The importance of soil properties for plant growth has been well understood since at least the beginning of the 19th century (Liebig 1840) and has resulted in 'the law of the minimum'. A nice illustration of this law is given by Tamm (1991, Fig. 4.8), who shows that relieving trees of N limitation rapidly leads to P limitation. When both N and P are available in amounts that are non-limiting for growth, other elements (K, Ca, Mg …) become limiting. There is a wealth of literature on the relationships between soil and plant properties, as well as on the circulation of individual elements (e.g. Attiwill and Adams 1993; Casper and others 2000; Gallardo and Covelo 2005), although predicting forest stand productivity from soil properties seems difficult (Jandl and Herzberger 2001). With the exception of C and N, little attention has been paid to the interactions between element cycles.

A classic notion in plant and ecosystem ecology is that organisms and their environments are intimately connected by exchange of chemical elements and that there is reciprocal control of elemental composition (for example, Redfield 1958; Odum 1971, p.23). Plants and other living organisms must control their internal chemical balance within certain ranges that normally differ from the chemical composition of the external environment (Ågren and Bosatta 1998), which can vary greatly in space and time. Changes in the chemical composition of a plant can be a consequence of changes in function. Different elements should then change in concert and the effects should be apparent as correlations between element concentrations in organisms and their environment, reflecting control of biogeochemical cycles in ecosystems. Changes can also occur through the plant responding passively to changes in the environment. In that case no relationships between elements can be expected.

The biogeochemistry of different elements differs greatly due to the degree of biological control, chemical bonding properties and the source of the elements. Biogeochemical pathways of N, C, and S are similar because biological redox reactions are major controlling processes, gas phases are important and they all form organic molecules with covalent bonds. However, S has both mineral (weathering) and atmospheric (volcanic) origins in ecosystems. Phosphorus also forms covalent bonds in organic matter but has no gas-phase or redox reactions of importance in biogeochemical cycles and originates only from minerals. The metal macronutrient cations originate only from minerals and show varying bonding properties. Potassium occurs predominantly as an electrolyte in cell sap or as a positive counter-ion to negative surfaces (proteins, macro-anions), whereas Ca and Mg often occur bound in complex form in organic molecules (cell walls, chlorophyll) or are deposited as insoluble salts (oxalates) (Fink 1991; Frausta da Silva and Williams 2001). Other metal ions (Fe, Mn) show varying valence, which is used in their biological function as micronutrients. Furthermore, toxic elements such as Al may be abundant in the environment and must also be controlled by organisms.

The growth of plants is often limited by N or P supply. Changes in the availability of these elements are therefore accompanied by changes in function, in that increasing growth rates require increasing concentrations of these elements – the growth rate hypothesis (Sterner and Elser 2002; Ågren 2004), which also explains how these two elements are coupled to each other and to C. For example, photosynthetic rates respond strongly to foliar N concentration in many species (e.g. Field and Mooney 1986; Reich and others 1997). The mechanisms behind stoichiometric couplings for other elements are less well understood or not understood at all. The uptake of N and P is also associated with high costs, so these elements normally should not be taken up in large excess. In contrast, although plants have many ways of controlling their internal, physiologically active nutrient composition, in general they take up non-limiting elements in concentrations exceeding not only current physiological needs but also the need for maximum growth rate (Knecht and Göransson 2004). Because such excess uptake is poorly regulated, the element concentrations can be highly variable and contain little information, which appears as noise in data. It is possible

that the uptake of some micronutrients proceeds up to some rather well-defined maximum concentration, in which case this element would show small variability within a species. Overall, the variability associated with noise can be expected to be larger than that associated with function. Low variability should therefore indicate that an element is under biological control. As a consequence, we can expect the ratios between C, N, P, and probably S in plants to show moderate variation compared with other elements and the abundance of non-limiting elements in the environment to be reflected in the concentration in plants. For example, Ca concentration in the soil should determine Ca concentrations in plant foliage. Much of the variability in non-limiting elements can thus be attributed to noise, both in the plant and in the environment. Furthermore, because nutrient:C ratios vary with growth rate, expressing nutrient concentrations as ratios to N should show less variation than concentrations by dry mass or C. Thus, N should be a better indicator of physiological activity than C. Furthermore, low variabilities or high correlations in one study but not in others should be signs of site-specific properties rather than general ecosystem characteristics.

Because leaf litterfall constitutes a major proportion of total litterfall in many forests (e.g. Starr and others 2005; Saarsalmi and others 2007), the chemical properties of leaves should have a large impact on humus chemical composition. Similarly, the uptake of nutrients comes to a large extent from mineralization of humus. These two ecosystem components should therefore resemble each other stoichiometrically. However, there are confounding factors. Whereas leaves are part of one organism with homeostatic regulation, humus results from the work of many different organisms. Litter input other than leaves of the dominant tree species, such as roots and mycorrhiza growing in the humus and root exudates, may add elements in other proportions, although it seems that different species at the same location have similar stoichiometry (Reimann and others 2001) and that nutrient concentrations covary within a species (Newman and Hart 2006). The differential, and interannually variable, retranslocation of elements in leaves during senescence also alters their proportions between the living leaves and what becomes litter (e.g. Killingbeck 1996; McGroddy and others 2004). These two factors should tend to make the stoichiometry in humus more variable than in leaves. On the other hand, the systematic changes in at least N/C and P/C, with a convergence towards similar ratios independently of the origin of litter material (Melillo and others 1989; Moore and others 2006), should drive down the variability in humus. The biological mineralization of N and biochemical mineralization of P (McGill and Cole 1981) could also make these elements behave differently. It is not clear whether the dominant forces in humus should act for more variability or less.

There may also be a difference between macro- and micronutrients. Wood and others (2006) observed a greater variability in micronutrient concentration than in macronutrient concentration in leaf litters at different sites in Costa Rica. Similar results, although N, P, and S were missing, were reported by Watanabe and others (2007) when analyzing leaf mineral concentrations in 670 species collected world-wide. The reason why micronutrients should be more variable than macronutrients is unknown.

In the present study, we analyzed a data set containing element concentrations in the forest floor and one-year old needles of *Picea abies* and *Pinus sylvestris* forest stands distributed across Sweden. These species are the major tree species of Sweden, ranging from the northern margin of boreal forests to the temperate zone in the south, predominantly growing on acid, podsolic soils. The study was restricted to the compartments one-year old foliage as representative of the physiological activity of the trees, and the humus layer as a product of both the underlying soil and tree litter.

Based on the above, we formulated the following five hypotheses:

1) Because biological processes control all transformations of C and N, these elements will show strong correlations within and between compartments (plant, soil).

2)   Because biological processes control transformations of C, N, P, and S, these elements will show less variability than other elements.
3)   Because the properties of humus are determined by the interaction of many organisms, the biologically controlled elements (C,S,N,P) should be more variable in humus than in needles.
4)   Because large fractions of C (and O and H) contribute solely to structure in plants, expressing element concentrations relative to N should capture more of their function and therefore be less variable and more correlated in plants. This difference between C and N should be absent for humus.
5)   The variability in macronutrients (N, P, K, Ca, Mg) in plants and in humus should be lower than that in micronutrients (for example Fe, Mn).

Materials and Methods

The study utilized monitoring data for forest stands from the Swedish database for ICP Forests (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests). The aim of the program is to monitor the effects of air pollution and climate change on forest ecosystems across Europe. In Sweden, the ICP Forest - Level II Programme has been operated by the Swedish Forest Agency since 1995.

Study area

Sweden ranges between latitudes 55° and 69° N and the study sites included here were located between 55°66'N and 66°07'N, the latter close to the Arctic Circle. The 60°N latitude approximately divides Sweden into a northern boreal zone with continental climate and cold winters (growing season of >5 °C 150-180 days, annual mean temperature 5 °C or less) and a southern hemiboreal-temperate zone with a more maritime climate (growing season 180-220 days). Only the coastal provinces of southernmost and SW Sweden have a temperate climate (mean annual temperature≥ 6 °C). Annual precipitation ranges between 600 and 800 mm in most parts of Sweden except for western and south-western provinces, which receive 800-–1000 mm annually or more (Raab and Vedin 1995).

Due to the proximity to the North Atlantic and prevailing SW winds, forests in SW Sweden have a greater input of sea salts than other regions of Sweden. To the east the Baltic Sea and, in particular, the Bothnian Bay are brackish waters.

Conifer forests of Sweden are dominated by podsols, with a mor humus layer overlying till or glaciofluvial sediments. The acidic character of the soils is largely due to slowly-weathering minerals originating from acid silicate bedrocks. However, in regions with warmer climates and lime-rich soils, moder or mull humus is frequent.

Monitoring plot design, sampling and chemical analyses

In 1995-1997, the Swedish Forest Agency established observation plots for the ICP Forest Programme. A detailed description of the methodology is given by Anonymous (2000, 2006) and is also published on the internet: http://www.icp-forests.org/Manual.htm. Plot selection was started by setting a national grid of 5 km x 5 km, in which the potential plot was located within a circle of 500 m radius from the centre of each square. The plots had to meet a number of criteria with respect to stand age (40-70 years in S Sweden, 60-90 years in N Sweden), tree species (the target tree species of the plot should dominate by at least 70%) and soils (excluding peat soils, lithosols, and rendzina). Furthermore, the stands were selected in order to minimize the influence of liming, fertilization, field drainage, traffic, agriculture (300 m), sea spray (4 km from coast) and emissions from industry (10 km). Maximum altitude was set to 500 m. Each monitoring plot is 40 m x 70 m and includes two quadratic subplots and a border zone where soil and needle sampling are carried out.

Sampling of needles was carried out during the period 1-15 August (i.e. when shoot development had ceased and before autumn senescence). A composite sample of each plot

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was made from 10 shoots sampled from the upper part of the canopies of at least 5 representative and dominant trees. Fresh samples were dried at 40 °C over 3-4 days, and were kept cool and dry until chemical analysis.

Two composite samples of approx. one l each were taken from the humus layer in each monitoring plot, and the samples were analyzed separately. Each sample was made by pooling 15-20 humus cores. After removal of living materials (such as mosses, roots, etc.) and stones and woody objects > 2cm, collected samples were transported to laboratory and dried at a temperature of 40 °C. Dried samples were sifted to size < 2 mm prior to chemical analyses. Plot mean values were calculated from separate analyses of the two samples.

The soil variables determined included total content of C and N (by dry oxidation method), total P (by wet oxidation in aqua regia followed by ICP-AES analysis), pH (water-extractable) and salt-extractable cations (0.1 M BaCl followed by ICP-AES analysis). Plant-essential nutrients were analyzed (macro-elements: N, P, K, S, Ca, Mg, microelements: Mn, Fe) along with non-essential nutrients (Na, Al). Element concentrations in needles per unit mass were determined by Kjeldahl wet digestion (N) and wet digestion followed by ICP-AES for other elements. Because the aim of the observation plots was to monitor forest status in relation to air pollution, which is most pronounced in SW Sweden, the density of the plots decreased from southern to northern Sweden. The monitoring plots thus represent the full range of conditions in Sweden, except for alpine forests.

We used observations from 1995, 1996, and 1997 for plots dominated by Norway spruce [*Picea abies* (L.) Karst.] (50 plots) or Scots pine (*Pinus sylvestris* L.) (37 plots) (Figure 1). The age of the stands studied ranged from 37 to 95 years, with 72% aged between 40 and 65 years. For the present study, we selected general site variables, needle variables, and soil variables. The general site variables included the Swedish site index H100 (SI), which measures the height of dominant trees at age 100 years (Hägglund and Lundmark 1977), latitude, longitude, and altitude. We selected the one-year old needle cohort data for foliage analyses, since this age class is considered the most reliable for diagnostic purposes (Linder 1995), and humus because it was expected to be the most responsive of soil variables. Figure 2 shows general characteristics of the stands included in this study.
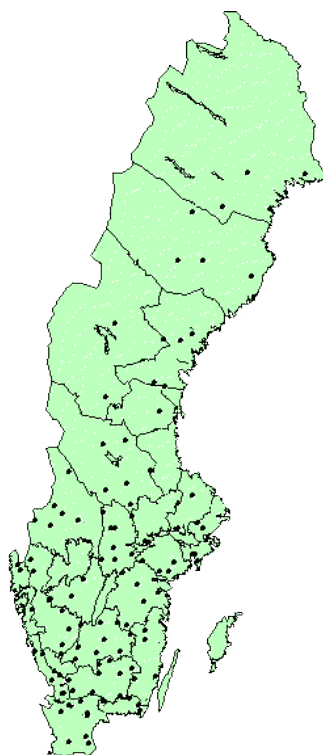
Figure 1. Approximate location of the sampling plots.
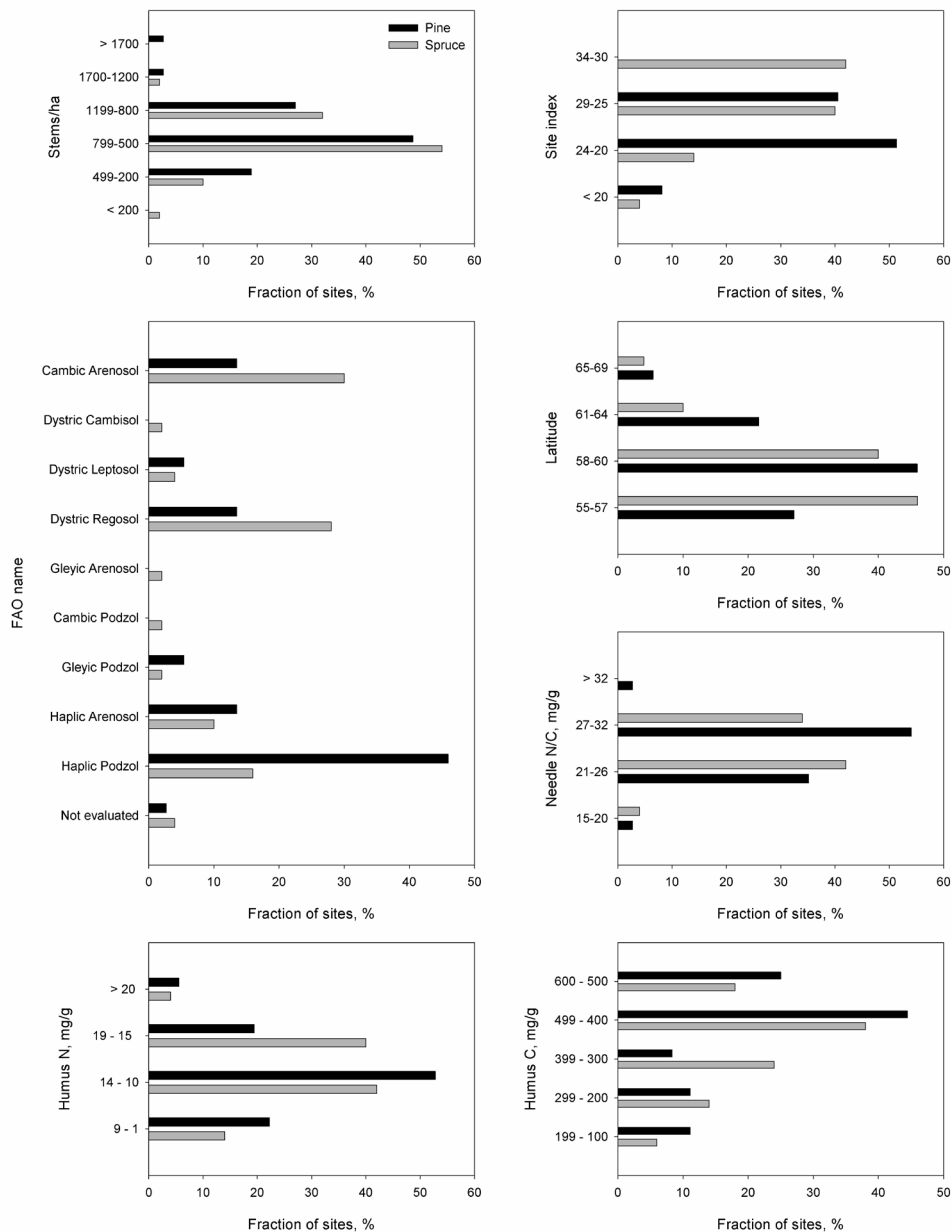
Figure 2. General characteristics of the investigated sites.

Data analysis

We called the variables obtained from the measurement program 'basic variables'. We also expressed element composition as the ratio of element concentration to C concentration or to N concentration. Carbon concentration in needles was not measured but was assumed to be 50%, the exact value not being critical as long as the concentration is the same across samples (own unpublished data; see also Perakis and others, 2006). The variables examined are presented in Table 1.

Coefficient of variation (CV = standard deviation/mean, %) was used to express the variation in soil and needle variables. We applied box plots to describe the CVs by the median and interquartile range (IQR). Pearson's rank correlation coefficients were calculated for a range of combinations of variables, while scatter plots were used to display data and to detect non-linearities in relationships. The data matrix obtained contained 1226 analyses of the 22 needle variables and 25 soil variables. Correlation coefficients larger than 0.32 for pine and 0.27 spruce were considered statistically significant ($P<0.05$).

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Table 1. List of variables.

Needle and humus variables	Abbreviation	CV, %			
		Pine		Spruce	
		Needle	Humus	Needle	Humus
Basic variables					
Hydrogen ions extractable	H^+		41		49
Aluminum	Al		44		87
Sodium	Na		61		69
Organic carbon (total)	C-tot		33		29
Organic nitrogen (total)	N-tot	14	38	11	29
Phosphorus (total)	P-tot	13	20	18	26
Sulfur	S	10		11	
Potassium	K	31	49	23	45
Calcium	Ca	38	45	25	54
Magnesium	Mg	12	55	13	42
Iron	Fe	18	92	24	124
Manganese	Mn	32	85	31	106
Nitrogen scaled variables					
Carbon nitrogen ratio	C/N		17		13
Phosphorus nitrogen ratio	P/N	10	62	22	42
Sulfur nitrogen ratio	S/N	10		12	
Potassium nitrogen ratio	K/N	33	59	28	46
Calcium nitrogen ratio	Ca/N	42	64	27	60
Magnesium nitrogen ratio	Mg/N	18	72	19	33
Iron nitrogen ratio	Fe/N	15	82	26	122
Manganese nitrogen ratio	Mn/N	35	108	30	132
Carbon scaled variables					
Nitrogen carbon ratio	N/C	14	17	11	13
Phosphorus carbon ratio	P/C	13	80	18	43
Potassium carbon ratio	K/C	31	49	23	41
Calcium carbon ratio	Ca/C	38	75	25	56
Magnesium carbon ratio	Mg/C	12	95	13	34
Iron carbon ratio	Fe/C	18	86	24	133
<u>Manganese carbon ratio</u>	Mn/C	32	109	31	137

List of variables with abbreviations used in figures and tables and their coefficients of variation (CV) for humus and needle variables in pine and spruce stands. CVs for element/carbon ratios in needles are by definition equivalent to element concentrations in needles but are included in the table for easy comparison with element/carbon ratios in humus

Results

Our analyses focused on identifying variables that provided examples of control and relationships. Controls were deduced from low variability and relationships from high correlations. However, it should be borne in mind that because of the large number of correlations calculated, there was a risk of significant correlations appearing by pure chance.

Variability in needle and soil properties

As shown by Table 1 and Figure 3, which present the CVs for 22 needle variables and 25 humus variables, there was a large range in CV, from 10 to 137%. However, there was a striking difference between the rather small variability of around 20% in needle variables and the larger variability of around 60% in humus variables. In contrast, the differences in variability between species were small for both needles and humus, although the variability in pine humus was over a narrower range than for spruce humus.

The variability in the needle variables increased in the following order:
S, S/N, P/N < Mg < P < N < Fe/N < Fe, Mg/N << K < Mn < K/N < Mn/N < Ca < Ca/N for pine; and

N, S < S/N< Mg < P < Mg/N < P/N < K < Fe < Ca < Fe/N < Ca/N < K/N < Mn/N <Mn for spruce.

Thus, the variability increased in approximately the same order for the two tree species. However, whereas there were possibly two groups of elements in pine, one with low variability (S, Mg, P, N, Fe) and one with high variability (K, Mn, Ca), the variability increased smoothly in spruce. Expressing nutrient concentrations in needles on a dry matter basis or relative to N had no effects on the ranking of variabilitites.

The variability in the humus variables increased in the following order:
N/C < C/N < P < C < N < Al < Ca < K, K/C < Mg < K/N < Na < P/N < Ca/N < Mg/N < Ca/C < P/C < Fe/N < Mn < Fe/C < Mg/C < Fe < Mn/N < Mn/C for pine; and
C/N, N/C < P < C, N < Mg/N < Mg/C < K/C < P/N, Mg < P/C < K < K/N < Ca < Ca/C < Ca/N < Na << Al < Mn < Fe/N < Fe < Mn/N < Fe/C < Mn/C for spruce.

As for the needles, the variability in humus variables increased in approximately the same order for the two species. However in contrast to the needles, the variability changed smoothly for pine humus variables but possibly separated into two regions (C, N, P, Mg, K, Ca, Na; and Al, Mn, Fe) for spruce humus.

There was also a coupling, albeit weak, between variability in needles and humus such that element concentrations that varied greatly in needles tended to do so also in humus (Figure 4). When the species were considered separately the correlation was absent for pine but stronger for spruce. The choice of basis C or N in ratios had no importance for the variables.
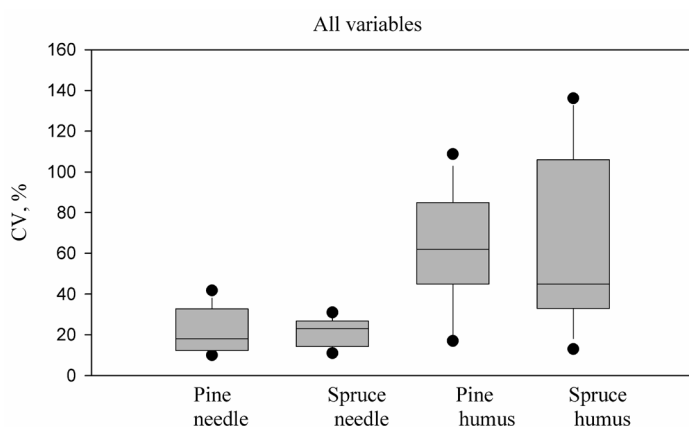

Figure 3. Box plots of coefficients of variation (CV, %) of needle and humus variables in pine and spruce stands. The box plots show the median, 75th, and 25th percentiles (box), and the highest and lowest observations (solid circles).
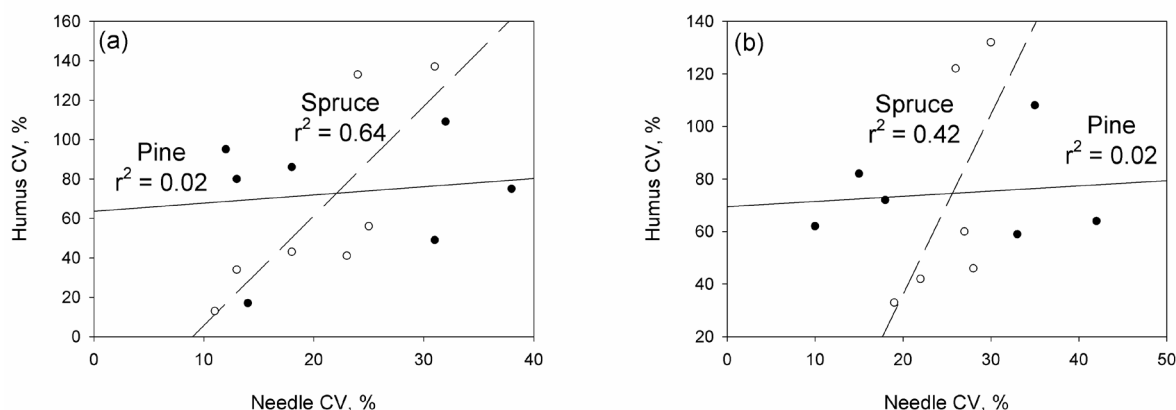
Figure 4. Correlations between humus CV and needle CV for: a) element to carbon ratios (N/C, P/C, K/C, Ca/C, Mg/C, Fe/C, Mn/C); b) element to nitrogen ratios (P/N, K/N, Ca/N, Mg/N, Fe/N, Mn/N). Pine: solid line and filled symbols. Spruce: broken line and open symbols.
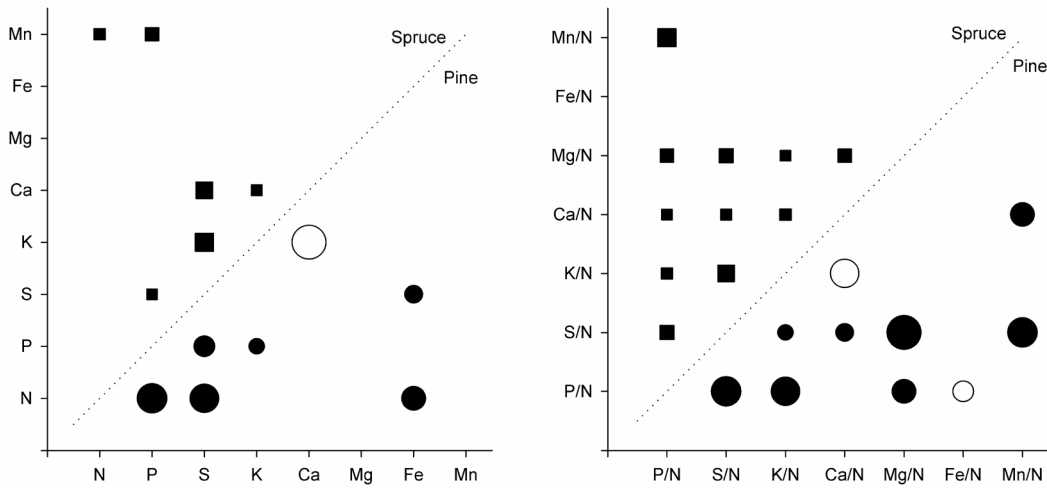
Figure 5. Correlation coefficients between concentrations in needles. The widths of the symbols are proportional to the magnitude of the correlation coefficient. Solid symbols represent positive values and open symbols negative values. (Left) Basic variables. Squares (upper left) are for spruce, range 0.29-0.50. Circles (lower right) are for pine, range 0.38- (-)0.82. (Right) Element/N ratios. Squares (upper left) are for spruce, range 0.32-0.71. Circles (lower right) are for pine, range 0.34-(-)0.65.
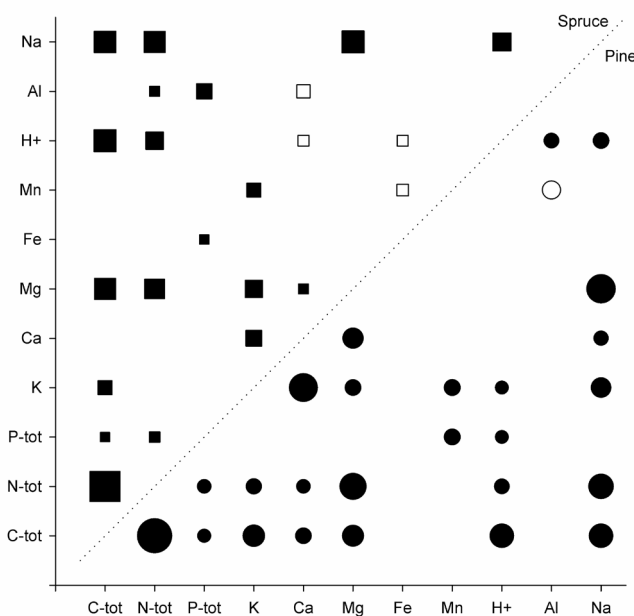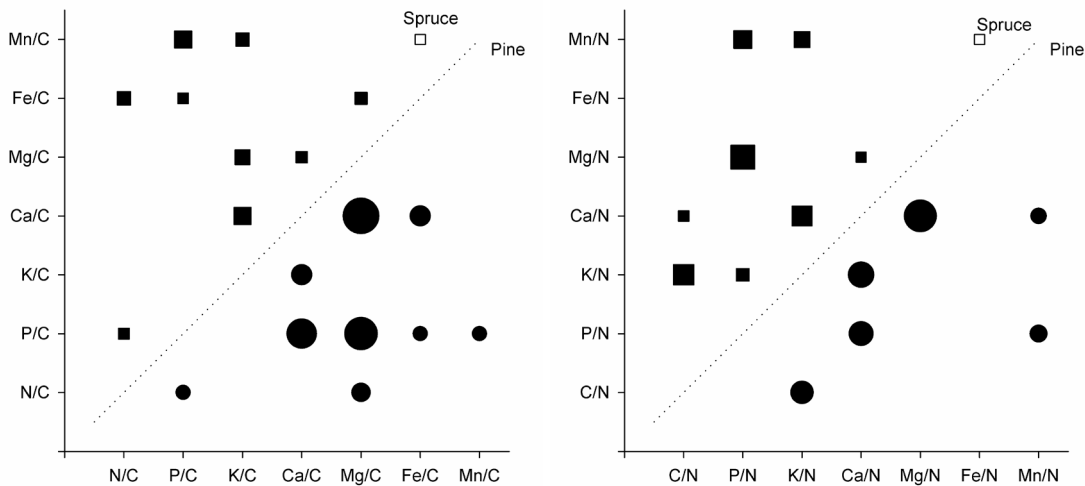

Figure 6. Correlation coefficients between element concentrations in humus. The widths of the symbols are proportional to the magnitude of the correlation coefficient. Solid symbols represent positive values and open symbols negative values. Squares (upper left) are for spruce, range 0.28-0.90. Circles (lower right) are for pine, range 0.34-0.89.

Correlations between variables

Variables that had at least one significant correlation coefficient with another variable are shown in Figures 5-9. Overall, the fraction of significant correlations was around 20-30% but could be as low as 10% (between needle variables and humus variables in pine stands) and as high as 47% (between humus variables in spruce stands).

Climate, as reflected by geographical location, had a strong influence on productivity, and site index was negatively correlated with latitude (r = -0.66 for pine and r = -0.85 for spruce). This was also reflected in correlations between needle N concentrations and site index (r = 0.71 for pine and r = 0.46 for spruce); data not shown. We did not pursue the question of relationships between stoichiometry and productivity any further in this study.

Figure 7. Correlation coefficients between element/C (left) and element/N (rigth) ratios in humus. The widths of the symbols are proportional to the magnitude of the correlation coefficient. Solid symbols represent positive values and open symbols negative values. Squares (upper left) are for spruce, range (-)0.28–0.66. Circles (lower right) are for pine, range 0.35–0.86.
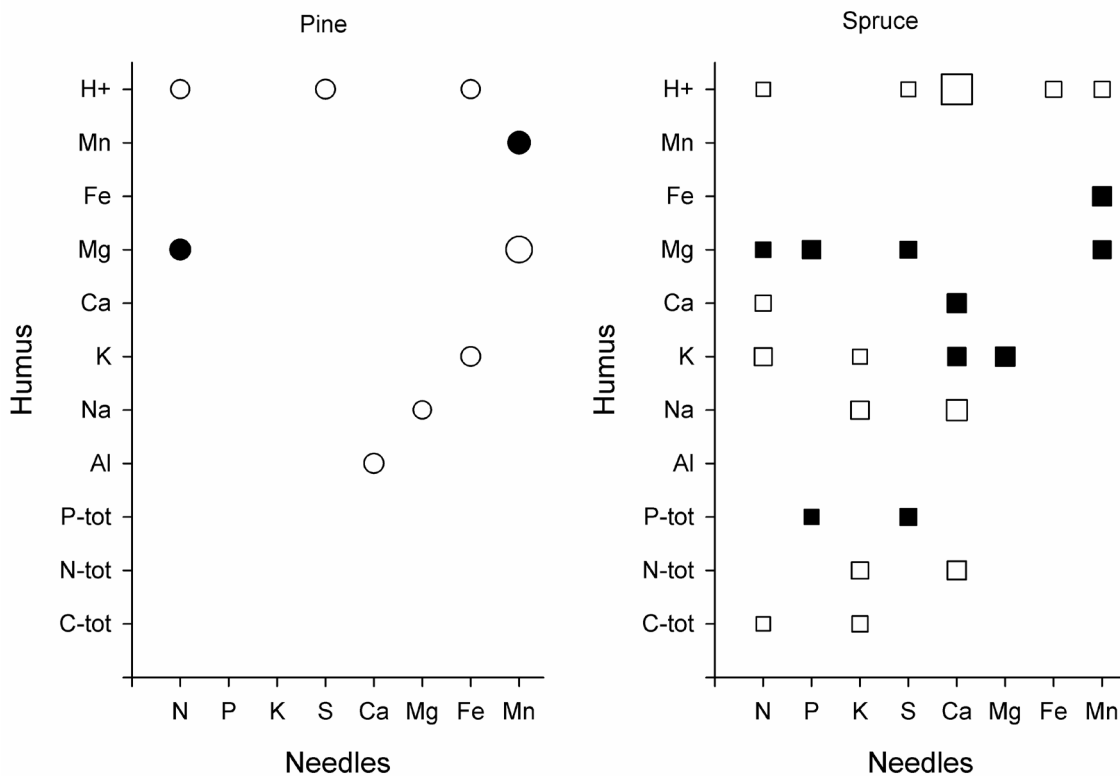
Figure 8. Correlation coefficients between element concentrations in humus and needles. The widths of the symbols are proportional to the magnitude of the correlation coefficient. Solid symbols represent positive values and open symbols negative values. Squares (right) are for spruce, range (-)0.28– 0.71. Circles (left) are for pine, range 0.34–(-)0.65.

Needle variables versus needle variables

In both stand types, only 25% of the 28 possible correlations between needle nutrients (expressed as basic variables) were statistically significant (Figure 5). The correlations were in general stronger in pine than in spruce. All the correlations involved the organically bound

elements, except that between K and Ca, which was negative in pine and positive in spruce. In particular, Mg did not correlate with any of the other elements. When element concentrations were expressed relative to N, the number of significant correlations increased to 48% of those possible for pine and 52% for spruce. In this case too, correlations were dominated by variables involving organically bound elements (P/N and S/N). When Mg was expressed relative to N (Mg/N), it correlated with several of the other variables, but Fe and Mn were still mostly uncorrelated to other elements (Figure 5).

Humus variables versus humus variables
There were 26 (47%) significant correlations among humus basic variables out of 55 possible correlations for pine stands and 23 (42%) for spruce (Figures 6-7). The strongest correlation was between humus C and N (+0.89 for pine and +0.90 for spruce). Organically bound elements were involved in many of the correlations but there was also a large number of correlations between the inorganic elements. Almost all of the correlations were positive. The possible number of correlations did not increase when element/C ratios rather than basic variables were analyzed. In contrast, the number of correlations decreased for pine when N was used in ratios (Figure 7). It should be noted that the negative correlation between Mn and Fe in spruce humus was entirely attributable to a few extreme values of low Mn and high Fe.

Needle variables versus humus variables
There were 9 (10%) significant relationships between humus and needle basic variables out of 88 possible for pine and 24 (27%) for spruce (Figure 8). In general, the correlations between needle and humus variables were also weaker in pine stands. The correlations in this case were dominated by variables where no organically bound elements were involved. There was also a tendency for negative correlations to dominate. Changing to element/C did not increase the number of correlations but converted them to mostly positive values (Figure 9). Using element/N ratios increased the number of significant correlations and converted all but one to positive values. N/C in humus and needles was one of two combinations of variables that correlated strongly in both pine and spruce stands, the other (which was probably a coincidence) being between Mg/N in needles and C/N in humus (Figure 10).

Discussion
This study focused on the relative strength of control of different element cycles and the interactions between element cycles in forest ecosystems. We approached this question by comparing two different species (*Pinus sylvestris* and *Picea abies*) and two ecosystem components (needles and humus). The results comprised large numbers of coefficients of variation, where small coefficients were taken to signify control and a large number of correlations as indicating how strongly elements and components were coupled. As far as we are aware there is only one comparable study, made in Finland on similar plant/soil components and with the same two species (Merilä and Derome 2008). Other studies (Huntington and others 1990; Bauer and others 1997; Hallett and Hornbeck 1997; Perakis and others 2006) examined narrower aspects of the issue. This study has resulted in a number of points that can be attributed to each of the aspects investigated. We emphasise these by setting them out in italic in the text.

Variability in needle and soil properties
The variability in humus properties was considerably larger than that in needle properties (Figure 3), confirming our hypothesis (3) that there are more factors influencing humus than needles. The variability in humus and needles was also coupled (Figure 4), such that properties that varied greatly in needles also did so in humus, an effect most clearly seen in spruce. Together, this suggests that feedback mechanisms stabilizing the interactions between elements are exerted by the trees and not by the soil (humus).
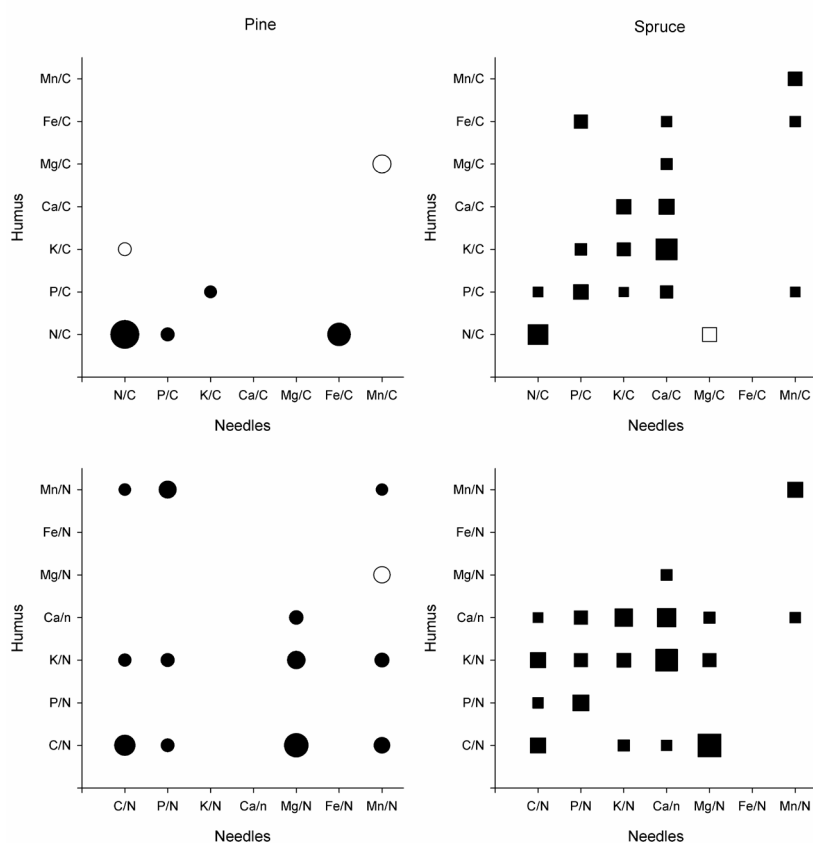
Figure 9. Correlation coefficients between element/C concentrations in humus and needles (top), respectively, element/N concentrations in humus and needles (bottom). The widths of the symbols are proportional to the magnitude of the correlation coefficient. Solid symbols represent positive values and open symbols negative values. Squares (right) are for spruce, range 0.29–0.68. Circles (left) are for pine, range 0.30–0.73.
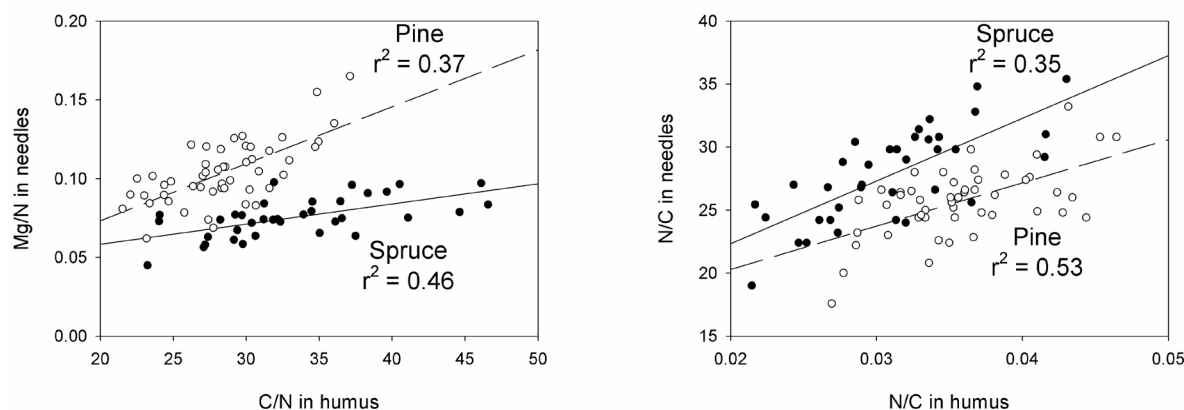
Figure 10. Correlations between soil and needle element ratios. Pine: solid line and filled symbols. Spruce: broken line and open symbols.

It was difficult to clearly distinguish groups of elements with clear differences in variability in our study. The elements N and P showed relatively small variability in both needles and humus. There was also small variability for S in needles and, although we lacked S data for humus, the variability was probably small as observed by Merilä and Derome (2008). At the other end, Mn was very variable, particularly in humus. Another group (K and Ca) had approximately equally high variability in needles and equally low variability in humus. In contrast, Fe and Mg had rather low variability in needles but high in humus. These results remained consistent when element variability was analyzed on the basis of concentration or as ratios to N, supporting our hypothesis 2 that C, N, P, and S should show low variability. However, there were other elements with low variability (Mg and Fe in needles, K and Ca in humus), which rejects our hypothesis 5 of a clear difference between macro- and micronutrients.

One possible cause for the great variation in the micronutrients Fe and Mn in humus,

unlike the macronutrient base cations, is that they are subject to oxidation (+III) and reduction (+II), which together with pH influence the solubility of the elements in the organic layer. The extractable concentrations of Mn and Fe could therefore be expected to be higher in moist gleyic podsols and in the most acid soils, but no such tendency appeared in our data. Another potential cause for high variation in Fe and Mn in humus might be varying inclusions of mineral soil in humus, for natural reasons or from sampling artifacts. However, this explanation is unlikely because the concentrations of Mn and Fe per unit dry weight are generally lower in the mineral soil than in the organic layer.

Our patterns of variability are similar to those found by Merilä and Derome (2008), the major difference being that our variability was higher overall for both needles and humus. This might be an effect of our larger sample, which could lead to larger variability in environmental factors – we had 50 spruce and 37 pine stands, compared with their 22 and 31, respectively. There were also some specific differences, e.g. we found a considerably larger variability in needle K and Ca concentrations.

When we extended the comparison of variability in leaves to a broader set of data reported in other studies, some other patterns emerged whereby S, N, P, and Mg formed a group with low variability even when calculated over several species and large geographical regions (Table 2). Calcium, Zn, and Mn formed another group with intermediate variability and Fe was alone in a group with high variability. Potassium seemed to fall between the first and the second group. This partitioning into groups has to be regarded with some caution, as the number of studies is small. These results support our hypothesis 5 of a difference between macro- and micronutrients. A closer look at the data reveals that much of the extreme variability in Zn and Fe is the result of a few observations; e.g. the Fe concentration in the mosses analyzed by Reimann and others (2001) was about five-fold higher than that in the other seven species studied. The capacity of mosses to accumulate high concentrations of metals has made them important indicator species (e.g. Selinus 1996). We suggest the following interpretation. Most species keep a tight control over both macro- and micronutrients, so that small CVs are found when a single species is studied over a geographical range, i.e. rejecting our hypothesis 5 of a difference between macro- and micronutrients. However, there are some species that can accumulate large quantities of micronutrients and in studies covering several species large CVs are found when these hyperaccumulating species are included in the sample, supporting the hypothesis 5. The validity of this hypothesis therefore depends on the context.

Correlations between needle variables
Several correlations were observed between element concentrations in the needles, all of which were positive except K versus Ca in pine (Figure 5). The correlations were dominated by those involving organically bound elements, with those in pine even being almost exclusively restricted to these variables. Somewhat surprisingly, S emerged as the element with the largest number of couplings to other elements. In pine, but not spruce, N and P were the elements most strongly coupled to each other, which agrees with the suggestion by Knecht and Göransson (2004) that the uptake of these two elements is the most strongly regulated. It should also be noted that with the possible exception of Mg, the macronutrients were correlated, whereas the micronutrients Fe and Mn were almost unrelated to the other elements, suggesting a stronger control of macronutrients in trees. Both Fe and Mn were taken up in excess of requirements for growth and the average ratios between the concentrations of Fe and N and Mn and N were 3.6 and 62 mg g^{-1}, respectively. Linder (1995) suggests that for optimal growth of spruce these ratios should be 2 and 0.5 mg g^{-1}. Judging by the low variability in Fe/N, the uptake of Fe seems to be controlled so that no large excesses build up. In contrast, uptake of Mn exceeds requirements by two orders of magnitude and the variability in Mn/N is large, suggesting that the uptake of Mn is mainly driven by availability.

Table 2. Comparison of CVs

	S	N	P	Mg	K	Ca	Zn	Mn	Fe
Pinus sylvestris[a]	10	14	13	12	31	38	-	32	18
Picea abies[a]	11	11	18	13	23	25	-	31	24
Pinus sylvestris, Finland[b]	9	12	10	14	7	20	12	29	-
Picea abies, Finland[b]	10	14	13	12	12	18	21	28	-
Picea abies, Europe[c]	24	22	33	54	23	51	-	-	-
Fagus sylvatica, Europe[c]	16	7	38	45	48	36	-	-	-
Metrosideros polymorpha, Hawaii[d]	-	20	27	49	26	51	-	-	-
Pseudotsuga menziesii, Oregon[e]	9	16	20	21	17	38	14	59	78
Mixed species, Costa Rica, inceptisol[f]	11	24	18	18	37	23	39	57	287
Mixed species, Costa Rica, ultisol plateau[f]	15	23	15	23	38	20	48	33	354
Mixed species, Costa Rica, ultisol slope[f]	12	27	14	16	29	14	46	47	107
Mixed species, northern Europe[g]		-	53	58	51	45	-	65	132
Mixed species, global[h]	-	-	-	80	87	72	225	234	1044
Median	11	16	18	21	29	36	39	40	120
Average	13	17	23	32	33	35	58	62	256

Comparison of CVs in leaf element concentrations from different studies, sorted after increasing median
[a]*This study*
[b]*Merilä and Deorme (2008)*
[c]*Bauer and others (1997)*
[d]*Vitousek and others (1992)*
[e]*Perakis and others (2006)*
[f]*Wood and others (2006)*
[g]*Reimann and others (2001)*
[h]*Watanabe and others (2007)*
- = not observed

The choice of basis for expressing needle element content is important. Using N as a basis rather than dry mass or C increases the number of correlations, as suggested in our initial hypothesis (4). This indicates that from a stoichiometric perspective, N is a more important reference than C. The reason is probably that C compounds comprise the structure of plants as well as serving as storage compounds (for example, starch), and can therefore vary more relative to other elements that are required in more narrowly defined proportions in metabolically active processes.

The only sign of an antagonistic relationship between elements was the negative correlation between K and Ca in pine. However, this does not rule out the possibility that such relationships exist, because without them uptake of some elements might have been even greater. With the strong limitation from N that should exist in these forests (e.g. Tamm 1991), uptake of other elements can be inhibited without limiting growth.

There are few other studies with sufficient data to allow meaningful comparisons with our results. Merilä and Derome (2008) calculated correlations between N and the other elements and found N to correlate with both P and S in pine, in agreement with our results. In addition, they reported significant correlations for K, Ca, and Zn. For spruce, they only found a correlation with S. In the study by Bauer and others (1997), only N and S were correlated for spruce and Mg and Ca for beech, although the lack of correlation with N for beech might be explained by the narrow range of N concentrations observed and the small sample. In the Perakis and others (2006) study of *Pseudotsuga menziesii* in Oregon, N and S were again strongly correlated (r = 0.78), whereas other significant correlations were unrelated to those in

other published studies. Before further conclusions can be drawn, additional data sets must be analyzed.

Correlations between humus variables
Humus variables proved to be most strongly correlated among themselves, with almost 50% of the possible correlations being significant (Figure 6). In contrast to the case in needles, the biologically controlled elements (C, N, and P) did not dominate the number of correlations and correlations between the other elements were also frequent. We interpreted this as an indication of the importance of purely chemical exchange reactions in maintaining element balances in humus. Another indication is that many of the other elements are correlated with C, which determines CEC in humus. The correlation coefficient between C in humus and CEC in our data was 0.59 (data not shown).

As a consequence of the tight correlations between C and N in humus, 0.89 and 0.90 in pine and spruce, respectively, it made little difference whether correlations between elements in humus were calculated with C or N as the basis, in contrast to needle variables. A reason for this tight correlation could be that fresh litter is less variable with respect to element concentration than live tissue as a result of similar resorption proficiency (sensu Killingbeck 1996) during senescence. The correlation between C and P was considerably weaker, but in P-limited ecosystems C and P would be expected to become more tightly coupled in humus and C and N less so.

The correlations of the form X/C or X/N versus Y/C or Y/N in needles (Figure 5) and humus (Figure 7), respectively, showed little overlap, indicating that controls over elements differ between soils and vegetation.

Correlations between needle and humus variables
We found almost no correlations between needle and humus basic variables for pine; for spruce only 25% of those possible were significant (Figure 8). In spruce the concentration of an element in the humus was also reflected in the concentration of the same element in the needles, with the exception of Mg and Fe. Such a relationship was also observed by Merilä and Derome (2008) but for both pine and spruce. However, they do not report correlations between different elements. The lack of a strong coupling between vegetation and soil is not unique to our investigation. For example, Wood and others (2006) found in their study of Costa Rican rainforests that relationships between soil element stocks and leaf nutrient concentrations existed for only two (P, Mn), and possibly Ca, out of seven elements investigated. Correlations between foliar and soil Ca have been reported in several studies (Jandl and Herzberg (2001) for *Picea abies*; Huntington and others (1990) for *Picea rubens*; Perakis and others (2006) for *Pseudotsuga menziesii*; Hallett and Hornbeck (1997) for *Pinus strobus*). Correlations for other elements seem to be lacking or show no consistent patterns between studies, although we are not always sure which possible correlations have been calculated. Our hypothesis 1 that the relationship between needle nutrients and soil nutrients should be stronger for the biologically controlled nutrients was not supported by the data (Figure 8). This lack of correlation can partly be explained by noise created by the variable content of minerals in the humus samples. We circumvented this problem when we normalized with respect to C or N (Figure 9) but there were still few correlations in pine stands. The decoupling between living needles and their dead remains in the humus should in part result from retranslocation of nutrients in needles at senescence. While the nutrient concentrations in live needles vary with growth conditions, retranslocation works towards the lowest possible level, which seems rather species- and site-independent (Killingbeck 1996). However, the stronger the limitation from an element, the more efficient the retranslocation (see for example, McGroddy and others 2004), although this is not always observed (Güsewell 2005). Because N dominates as a limiting element, correlation ratios with N as a basis (for example, P/N in needles versus P/N in humus) can be expected to be weak because

the other elements will vary independently of N (the strong correlation for N/C is an exception). The existence of several correlations of this kind in spruce forests may be an indication that spruce forests are more susceptible than pine forests to becoming limited by elements other than N. Another explanation for the difference between species could be that pine forests in general are rather open, with important contributions to humus formation from ground vegetation, in contrast to dense spruce forests with no ground vegetation at all in some cases. A further decoupling between plants and humus can occur because the plants can take up elements both from the mineral soil and from deposition. However, these elements will be returned to the humus where they also can be leached from the system.

Concluding remarks

We formulated five hypotheses about element relationships in forest ecosystems, some of which were supported entirely or partially by the data, whereas others were rejected. Elements that are covalently bound and coupled to biological processes for transformation were clearly less variable than other elements, but only when considered within needles or humus (hypotheses 1 and 2). They also appeared in stricter relationships with each other. The control over S was tighter than expected. However, the stoichiometric relationships were not entirely rigid. Although it is clear that there are some minimum relationships between elements that need to be satisfied, there is limited knowledge about their quantitative nature (Ågren 2008). Further progress requires better understanding of the causes and costs of excess nutrient uptake by plants and why the uptake is limited although more is available for uptake in the environment. The strong correlation between N and Fe in pine needles could be an example of this. This relationship between N and Fe shows, however, that the biological control is not limited to the covalently bound elements. Copper, although not included in our analysis, might be another example (see Wood and other 2006). Overall, we found that macronutrients were no less variable and more controlled than micronutrients rejecting hypothesis 5.

Although humus is formed by biological processes, it is also subject to chemical exchange reactions. The biological control over covalently bound elements changes from tree properties in the youngest components to decomposer properties in the older parts. Because plants and decomposers have different stoichiometry, this mixture is less well defined than its end components, fresh litter and decomposers. This explains why biologically controlled elements exhibited higher variation in humus than in needles (hypothesis 3). On the other hand, humus as a substrate for exchange reactions seems less variable, which can explain why relationships between cations were similar, independent of site.

It must be emphasized that the basis chosen in stoichiometric relationships is important. The use of N rather than C or dry weight (hypothesis 4) seems preferable.

We found strict control of the relationships between N and C in both needles and humus. There was also control, although weaker, of the relationships between several other elements, but some elements (e.g. Mn) seemed to be under no biological control.

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