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Parkano Research Station

Academic dissertation in
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– *Nullus est liber tam malus, ut non aliqua parte prodesset* –

Parkano, November 2002,

Päivi Merilä

Original publications

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I Merilä, P., Lindgren, M., Raitio, H. & Salemaa, M. 1998. Relationships between crown condition, tree nutrition and soil properties in the coastal *Picea abies* forests (Western Finland). Scandinavian Journal of Forest Research 13(4): 413–420

II Merilä, P. & Ohtonen, R. 1997. Soil microbial activity in the coastal Norway spruce [*Picea abies* (L.) Karst.] forests of the Gulf of Bothnia in relation to humus-layer quality, moisture and soil types. Biology and Fertility of Soils 25(4): 361–365

III Merilä P., Smolander, A. & Strömmer, R. 2002. Soil nitrogen transformations along a primary succession transect on the land-uplift coast in western Finland. Soil Biology & Biochemistry 34(3): 373–385

IV Merilä, P., Strömmer, R. & Fritze, H. 2002. Soil microbial activity and community structure along a primary succession transect on the land-uplift coast in western Finland. Soil Biology & Biochemistry 34(11): 1647–1654

Päivi Merilä was responsible for the idea, preparation and writing of all the papers (I, II, III, IV). Rauni Strömmer (former Ohtonen) was the supervisor of this thesis and performed the soil microbial activity measurements (II, IV). Hannu Raitio was the coordinator of the research project under which Papers I and II were carried out, and was also responsible for the needle chemistry data (I). Martti Lindgren and Maija Salemaa were responsible for the crown condition data (I, II). Aino Smolander provided advice in performing the net N mineralisation experiments and in interpreting the results. Hannu Fritze performed the phospholipid fatty acid analyses and gave advice on their interpretation.

Abstract

Merilä, P. 2002. Soil microbial dynamics and the condition of Norway spruce on the Bothnian land-uplift coast. Finnish Forest Research Institute, Research Papers 877. 55 p. + 4 appendices.

The poor condition of Norway spruce (*Picea abies* (L.) Karst.) forests growing in the coastal area of the Gulf of Bothnia, western Finland, has been a cause of concern for several decades. In this study, the crown condition of spruce in the coastal area was compared with that of spruce growing in other parts of southern Finland. The variability in the crown condition of coastal spruce was evaluated in relation to foliar chemistry, soil type, and the mineral nutrient and moisture status of the organic layer in 30 forest sites. Relationships between the chemical, physical and microbial properties of the organic layer were also studied in a survey covering the same 30 coastal sites and 12 sites in the coastal region of Västerbotten, Sweden. None of the studied stands were growing on acid sulphate soils, which is a type of soil that occurs sporadically in the coastal region of the Gulf of Bothnia.

The spruce stands older than 60 years were more defoliated in coastal Ostrobothnia than in other parts of southern Finland. Defoliation and discoloration increased with increasing stand age. Old spruce stands that were strongly defoliated and discoloured also had low needle nitrogen and copper concentrations and the highest boron concentrations. Total nitrogen and extractable sulphur concentrations in the organic layer decreased with increasing stand age, and degree of defoliation and discoloration.

The most common soil types in the stands on the Ostrobothnian coast were carbic podzols and dystric gleysols, which develop in sporadically waterlogged soil conditions. Crown condition was found to be the poorest in old stands growing on these soil types. The carbic podzols and dystric gleysols also differed from the ferric podzols as regards certain microbial activities and the physico-chemical properties of the organic layer. The organic layer of the carbic podzols had lower basal respiration (BASAL) and substrate-induced respiration (SIR), and the gleysols had lower SIR than the ferric podzols. The results support the assumption that, especially on carbic podzols and dystric gleysols, poor nutrient status, acidity and a lack of oxygen due to sporadic periods of excess moisture in the organic layer, result in low microbial activity, impaired water and nutrient uptake and, consequently, poor condition of the spruce trees.

In this study, attention was also focused on successional changes in a forest ecosystem along a primary successional transect, located in the archipelago of Raippaluoto (Björkö and Replot; 63°20'N, 21°15'E). The transect represented a spatial continuum at right angles to the coastline as a result of ongoing post-glacial isostatic rebound (8 – 9 mm yr^{-1}). The transect comprised four forest sites: alder/rowan [70-year-old *Alnus incana* (L.) Moench/*Sorbus aucuparia* L.], birch (mainly 80-year-old *Betula pubescens* Ehrh.), birch/spruce [75-year old *B. pubescens* Ehrh. and *B. pendula* Roth./*Picea abies* (L.) Karst.] and spruce I (95-year-old *P. abies*). In order to extend the age sequence, a fifth forest site (spruce II; 130-year old *P. abies*) was chosen 12.2 km to the south of the transect.

Hypothesizing that a reduction in the availability of nutrients (especially nitrogen) during forest succession contributes to the poor condition of aged spruce crowns, I focused attention on the changes occurring in carbon (C)- and nitrogen (N)-related microbial

activities (net and gross N mineralisation, microbial biomass N, BASAL and SIR) in the organic layer along the successional transect. Phospholipid fatty acid (PLFA) analysis was used to detect concurrent changes in the microbial community structure.

The soil C/N ratio along the primary successional transect increased from 16 to 37, and the pH(H_2O) decreased from 5.1 to 4.0. Net N mineralisation decreased substantially. The young alder/rowan site was the only site to show net nitrification. BASAL and SIR remained mainly stable although, during the most favourable temperature and moisture conditions in the field, they tended to increase along the transect from the alder/rowan site to spruce I, and decreased again in spruce II. Microbial biomass N, measured once during the most favourable conditions in the field, also increased along the transect from the alder/rowan site to spruce I. Concurrently, gross N mineralisation showed a tentative increasing trend along the transect, although the differences between the sites were non-significant. The lower net N mineralisation in the spruce sites compared to the alder/rowan site was thus due to higher microbial immobilisation of N, rather than to a lower gross N mineralisation. It may also further be hypothesized that, in late successional spruce sites, a higher proportion of the N in the microbial pool will be further transformed to the more stabile N pool, i.e. to humic substances, resulting in a decreasing net N mineralisation along the transect. As shown by NMS (non-metric multidimensional scaling) ordination of the PLFA data, the microbial community structure showed clear differences along the transect and was closely related to the C/N ratio and pH of the organic layer.

The transect study provided evidence of distinctive changes in organic matter quality and decreasing availability of mineral N during forest succession. Low N availability may contribute to the poor crown condition and growth of the aged Norway spruce stands on the land-uplift coast in western Finland.

Key words: *Alnus incana*, *Betula sp.*, defoliation, discoloration, forest condition, forest soil, land uplift, needle analysis, nitrification, nitrogen mineralisation, phospholipid fatty acids, *Picea abies*, primary succession, soil fertility, soil respiration, substrate-induced respiration

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1. Introduction

1.1. Background

In the late 1970's and 1980's reports of decline in forest condition became headline topics in many parts of the world (Schütt & Cowling 1985, Huettl & Mueller-Dombois 1993, Innes 1993a). The deterioration in tree condition not only occurred in highly polluted areas close to industrial regions, but also in areas located at considerable distances from emission sources. In Central Europe the forest damage was initially characterized as needle loss and chlorosis in silver fir at high elevations, but reports of decline affecting a number of other species, including Norway spruce, Scots pine, and European beech, quickly followed (Augustin & Andreae 1998). Later on this phenomenon also affected oak species (Augustin & Andreae 1998). Long-range transboundary air pollution (primarily NO_x, SO_x, more recently also O_3) was suspected to be the main cause of the reported damages. This aroused widespread concern about the current state and future development of forest condition throughout the industrialised countries. These events prompted a considerable volume of research into cause-effect mechanisms, and highlighted the urgent need to follow, at regular intervals, possible changes in forest condition over large geographical areas. At the present time, forest condition is being monitored in ca. 30 countries using common methods in accordance with the UN/ECE recommendations (Müller-Edzards et al. 1997).

A number of multidisciplinary, regional forest condition surveys were carried out during the1990's in response to concern that the vitality of forests in Finland and in neighbouring areas may also be threatened (Tikkanen & Niemelä 1995, Raitio 1996, Lumme et al. 1997). This thesis originates from a survey conducted on the condition and growth of Norway spruce stands along the coastal areas of the Gulf of Bothnia (Raitio 1996). On the Finnish side of the Gulf, the issue was discussed as early as during the 1940's (Appelroth 1948) suggesting that natural factors rather than anthropogenic pollutants could be the primary cause of the poor crown condition of aged spruce stands in the area. In addition, several earlier studies (Kuusela 1977, Nyyssönen & Mielikäinen 1978, Tamminen 1993) indicated poor growth of coniferous stands in the coastal region of Ostrobothnia compared to other parts of southern Finland. However, more comprehensive ecological studies on the condition of coastal forests were lacking.

1.2. Forest condition vs. external appearance of the tree crowns

Forest condition - also synonymously termed as vitality, vigour and the health status of the forest - is a general expression and, consequently, vaguely defined. It has clearly been used in many different meanings, and often synonymously with defoliation and discoloration of the tree crowns. On the one hand, visual crown condition is considered as one of the most obvious indicators of the health status of forests (UN/ECE 2000), while, on the other hand, the relevance of defoliation and discoloration as forest health indices has also been criticized by several authors (Innes 1993c, Ferretti 1998, Helmisaari 1998).

The definition formulated by Andersson (1995) expresses the ultimate complexity of the concept in question: 'vitality is the ability of an organism to survive, grow and produce new generations when exposed to various stress factors: climatic factors, soil chemical factors, competition, consumers/ pathogens, air pollutants'. Functionally, the foliage is the assimilative apparatus of the tree, absorbing light energy and converting it into chemical energy, which is the physiological process on which all the other vital functions of the tree depend. A decrease in the amount of foliage biomass, or changes in pigment composition that appear as discoloration, can be assumed to reduce the photosynthesising capacity of the tree, which further impairs its other functions. Furthermore, disturbances in the uptake of water and nutrients, or their reduced/excess availability, are reflected in the biomass and physiological processes in the needles and, consequently, the appearance of the crown. These fundamental arguments have been used to rationalize the use of defoliation and discoloration as indicators of the general condition of the tree (Salemaa & Lindgren 2000).

1.3. Factors affecting tree crowns

Site conditions, spatial status and tree age greatly influence the external appearance of tree crowns. The aboveground biomass of trees is determined by climate and soil fertility, which can be defined as the ability of soil to provide plants with the nutrients they require (Kimmins 1987). Aboveground productivity in nutrient-poor sites is less than that in fertile sites, and a larger root system is needed to meet the water and nutrient demands of the trees (Keyes & Grier 1981). Within a forest stand, the size of the living tree crown is also greatly modified by competition for light. Crown defoliation is known to increase with increasing age (Thomsen & Nellemann 1994, Lindgren et al. 2000), although it is difficult to determine whether this relationship results from natural aging processes or whether older trees are more susceptible to

the stresses that promote defoliation (Innes 1993c).

The tree foliage is susceptible to a range of biotic agents (herbivores, pathogens), episodes of extreme weather conditions (wind, frost, drought, excess water, soil frost) and anthropogenic factors (air pollutants, harvesting damage), which may cause foliage loss or premature foliage fall, as well as discoloration of the crown. The effect of these factors is pronounced in evergreen, coniferous tree species that have a long needle retention time, such as Norway spruce. In a Finnish study on the dynamics and covariation of defoliation and biotic and abiotic damages of conifers during 1986-1998, the most marked change on the spruces was reported to be the increase in damage caused by *Chrysomyxa ledi* (Alb. & Schw.) deBary in 1988-1989 and the increase in frost injuries in 1993 (Nevalainen & Heinonen 2000). The overall contribution of damage to defoliation was, however, difficult to demonstrate.

The damaging effects of air pollutants on forest condition are strikingly evident around certain point sources, primarily energy production plants and metallurgical industries. The pollutants of primary concern around these point sources are sulphur dioxide, particulate and gaseous fluoride compounds, and numerous heavy metals (Smith 1990). Smith (1990) mentions that regional-scale air pollutants include ozone (and other oxidants), heavy metals and other trace metals (cadmium, cobalt, copper, lead, mercury, molybdenum, nickel, vanadium, and zinc), and acidic deposition (sulphuric and nitric acids). Of the anthropogenic air pollutants, the effects of mineral nitrogen compounds (ammonium and nitrate) are more complex, because nitrogen (N) is also the factor limiting productivity in many natural terrestrial ecosystems (Tamm 1991). Consequently, atmospheric N deposition can initially result in higher biomass production and increased nutrient uptake (Bauer et al. 2000). However, when so-called nitrogen saturation is reached, cronic N deposition has a number of adverse effects on forest ecosystems (e.g. van Breemen & van Dijk 1988).

Although harmful effects of air pollutants on forest ecosystems are indisputable (Smith 1990, Godbold & Hüttermann 1994), the degree to which these air pollutants contributed to the impaired crown condition in background areas in Europe and in North America in the late 1970's and during the 1980's is still a matter of controversy. In surveys conducted across Europe, a clear correlation between defoliation and air pollution was found only in Norway, although only a few countries could totally exclude long-range air pollution as a factor affecting crown condition (Müller-Edzards et al. 1997). Skelly & Innes (1994) clearly stated that 'connecting air pollution with the diverse symptoms of supposed forest declines over the last several decades is unjustified'. Impaired forest condition has, in many cases, been related to

nutritional disorders caused by natural soil properties, forest management, adverse climatic episodes (drought) and their interaction (Landmann 1992, Huettl 1993).

1.4. Indicators for assessing tree/forest condition

1.4.1. Defoliation and discoloration

By definition, the degree of defoliation is assessed as the relative leaf or needle loss in the crown as compared to a reference tree (UN/ECE 1998). The reference tree can be either a real, non-defoliated tree of the same age, same type of crown and growing under similar conditions in the vicinity of the sample tree, or an imaginary tree with a degree of defoliation of 0% (Salemaa & Lindgren 2000). When assessing the degree of defoliation, the influence of normal tree aging and its social status, as well as the effect of natural permanent site conditions on needle/leaf biomass, should be recognized and omitted. In order to avoid the effects of shading on tree defoliation, only predominant, dominant, and co-dominant trees without significant mechanical damage qualify as sample trees (UN/ECE 1998). The effect of natural pruning is excluded by limiting the assessment of Norway spruce to the upper half of the living crown. In the case of Norway spruce especially, the phenotypic branching type (comb, brush and plate types and their combinations) greatly influences the appearance of the crown and should to be taken into account while making the assessment.

Needle discoloration is defined as deviation from the usual colour of the living foliage of the species in question (UN/ECE 1998). The variation in the nature, extent and location of discoloration is a source of important diagnostic information since, in principle, many fungal diseases, nutritional disorders and exposure to certain air pollutants such as ozone, produce relatively clear visible symptoms (Skelly et al. 1987, Kurkela 1994, Marschner 1995). In practice, however, reliable diagnoses that are based solely on visible symptoms are often unsuccessful because the symptoms are not specific enough and several damaging agents may occur simultaneously. The description, and especially, the quantification of diverse symptoms, are problematic and limit the possibilities of further analysing the data. In order to make the visual observations consistent, repeatable and comparable between different observers and different observation years, comprehensive regular training is necessary (Salemaa & Lindgren 2000).

The implementation of the large-scale surveys of forest condition was primarily governed by practical considerations. Defoliation and discoloration of the tree crowns were chosen as the primary indicators of forest condition

because they were the most clearly visible symptoms of the observed forest damages (Schütt & Cowling 1985). The assessments of defoliation and discoloration can be carried out rapidly over large areas at low cost and without destructive sampling (Lorenz 1995). The value of defoliation degree as an indicator of the overall condition of the tree crown has proved to be reasonably good (Innes 1993b). However, both defoliation and discoloration of the crown are unspecific symptoms, caused by different processes induced by a number of factors. In order to make a precise diagnosis of the causes of the symptoms observed, additional information is undoubtedly needed.

1.4.2. Elemental composition of the foliage

Nutrient deficiencies, excesses and imbalances in plants can be diagnosed on the basis of the elemental composition of the foliage (Kimmins 1987, Walworth & Sumner 1988). Foliar analysis is a quick, relatively inexpensive diagnostic tool that is also suitable for large-scale monitoring purposes. Conventionally, the nutritional status of a tree or site is assessed by comparing the nutrient concentration in a foliar sample with a standard value or range for the nutrient in question (Ahrens 1964, Morrison 1974, Jukka 1988, Walworth & Sumner 1988). The essential importance of nutrient ratios was demonstrated by Ingestad (1971, 1979, 1981), who postulated that the optimum growth of higher plants was achieved when the ratios of macronutrients were ascertained to a certain range (N:K:P:Ca:Mg 100:50:16:5:5, respectively (Ingestad 1979)). Since nitrogen is frequently the limiting nutrient in boreal forest ecosystems, it is appropriate to consider the sufficiency of other nutrients in relation to the nitrogen concentration.

Because several factors cause considerable fluctuation in the elemental concentrations in needles, foliar diagnosis based on standard values and ranges should be considered as being merely tentative. The application of empirically derived standard values and ranges may be misleading since they are valid only in the conditions in which they have been determined (Timmer 1991). Rapid growth may result in a dilution effect, i.e. low concentrations of certain elements owing to the incorporation of carbohydrates in the biomass. The elemental concentrations in needles fluctuate according to variations in the dry matter content of the needles, which varies seasonally and increases with the age of the needles. In the study of Linder (1995), for example, most of the seasonal variation in the nutrient concentrations of Norway spruce needles could be explained by the variation in the concentration of starch in the needles. Therefore, sampling is generally recommended to be carried out during the dormant period. The increase in the dry matter content with spruce needle age results in a decrease in the element concentrations, with the exception of

Ca and Mn (Linder 1995, Raitio & Merilä 1998). The decrease in N, P, K and Mg with needle age may also, in part, be due to retranslocation of these nutrients (Meier et al. 1985, Helmisaari 1992, Marschner 1995). Finally, the root/shoot dry weight ratio generally increases as the nutrient availability decreases. This relationship is most obvious for nitrogen and less distinct for phosphorus (Marschner 1995). Decreased nutrient availability can also lead to reduced leaf size (Linder 1987). To some extent the foliage biomass is thus in balance with the supply of essential nutrients, while the nutrient concentrations in the needles remain relatively constant. Consequently, slight deficiencies may have minimum if any effects on the needle concentrations.

Bearing in mind the limitations of the method, determining the elemental composition of the foliage provides valuable information on the plant's overall nutritional condition (Walworth & Sumner 1988, Marschner 1995). In the case of surveys and monitoring studies, the comparability of the results is of extreme importance. Some sources of variation (seasonal variation, canopy layer, section of the crown, age of the needles, analytical errors) can be standardized by means of sampling design and consistent analysis (Raitio 1993).

1.4.3. Physical and chemical properties of the soil

Numerous physical and chemical soil analyses are available for describing the conditions in which plants grow: conditions for anchorage of roots, the supply of water, air (oxygen) and nutrients, and buffering against adverse changes in temperature and pH (Wild 1993). The physical properties of the soil, such as texture, structure, porosity and temperature, have a great influence on these basic necessities. Basically, the chemical properties of the soil (e.g. cation and anion exchange capacity, pH, and the forms and availability of nutrients) regulate the availability of nutrients to plants. The total amount and the "availability" of nutrients in the soil can be measured analytically. However, it is not clear which of the extraction methods that are used provides the most useful measure of the amount of available nutrients in different situations. Difficulties in estimating the amounts of plant-available nutrients are not only restricted to chemical aspects. All soils are characterized by extremely high spatial variability, and the error arising from field sampling is typically much larger than that associated with sample preparation, handling, or analysis (Crépin & Johnson 1993). In order to make accurate measurements of nutrient availability we should in fact extract the nutrients that are root available. In practice, however, the analyses are usually made on the bulk soil samples. The roots are able, by means of root exudates, to actively modify the chemical conditions, such as pH, in the rhizosphere, thus affecting nutrient

availability. Moreover, chemical soil analysis, being a one-off measure, does not take into account dynamics of the cycling of the nutrients.

The prevailing soil conditions results from the interaction of physical, chemical and biological factors. The upper part of the soil is exposed to soil formation processes, the course of which depend on the parent material, climate, topography, biota (mainly vegetation) and human activities. In the course of time, soil formation processes result in the development of characteristic horizons which, in boreal coniferous forests, typically means podzolization (Duchaufour 1982). Definition of the soil type may give valuable information about the prevailing conditions such as the long-term moisture status of the site.

1.5. The role of surface organic matter in nutrient dynamics of boreal forests

Boreal forest ecosystems are characterized by the accumulation of organic matter at the soil surface. The organic layer, also referred to as the forest floor, mainly originates from plant residues, consisting of above- and below-ground litter, and root exudates. The greatest microbial activity and highest density of nutrient-foraging roots are found in the organic layer (Van Cleve & Moore 1978). The physical and chemical composition, as well as the temperature and moisture conditions and the abundance and composition of soil microbial and faunal communities, are considered to be the key factors controlling the decomposition processes and, consequently, the type of forest floor that is formed (Kimmins 1987). Decomposition can be described as a two-phase process (Berg & Staaf 1980). The initial flush of decomposition is controlled by the climate and the concentrations of major nutrients and water-soluble organic compounds. The later, much slower phase of decomposition, is regulated by the decomposition of lignin compounds. The formation of stable humic substances further contributes to the retarded rate of decomposition.

In the boreal region, the slow rate of decomposition is primarily due to the cool, humid climate and the presence of relatively recalcitrant coniferous litter. In these conditions, the organic layer forms an important reservoir of carbon and nutrients; nitrogen (N), being the main limiting nutrient, regulates the site productivity. In undisturbed mature ecosystems, the supply of N is largely controlled by the rate at which plant-available N is produced from soil organic matter via decomposition, ammonification and nitrification (Tamm 1991). In addition to the rates of ammonification and nitrification, N availability to plants is also influenced by the rate at which inorganic N is consumed in microbial immobilisation. The mycorrhizal and non-mycorrhizal

uptake of organic N have also been demonstrated (Kielland 1994, Raab et al. 1996, Näsholm et al. 1998).

The organic matter in the soil makes many beneficial contributions to the stability of the forest ecosystem. It plays a vital role in the establishment of soil structure and in the maintenance of its stability. One important property of soil organic matter is that it improves the water-holding and cation exchange capacity of the soil. However, in conditions that are too cold, too wet or where the litter is unsuitable for faunal degradation, the progressive accumulation of organic matter on the surface of the soil leads to the immobilization of nutrients in the organic layer, paludification and a reduction in site fertility (Prescott et al. 2000).

1.6. The coastal chronosequence - a tool for studying successional changes

Due to isostatic rebound, the coastline along the Gulf of Bothnia between Finland and Sweden is continuously rising at a rate of 8–9 mm per year (Mäkinen et al. 1986). New land is becoming exposed to the combined effect of soil formation (Starr 1991) and other ecosystem processes that are controlled by the prevailing climate. The successional stages of the forest ecosystems thus appear as a spatial continuum running at right angles to the coastline (e.g. Ericson 1982, Svensson & Jeglum 2000). The succession of forest vegetation on stony, fine-textured till soils starts from alder-dominated (*Alnus incana* (L.) Moench) deciduous shoreline vegetation, and ends in almost pure Norway spruce stands (Appelroth 1948, Svensson & Jeglum 2000). On gently sloping shores the succession sere also includes a birch-dominated (mostly *Betula pubescens* Ehrh.), intermediate stage (Svenonius 1945). The ecological change from the dinitrogen-fixing alder stage to the frequently paludified, nitrogen-deficient spruce stands with a thick humus layer is considerable. A chronosequence of this kind offers an opportunity to study the inter-relationship between vegetational succession and the microbial processes that affect organic matter decomposition, N transformations, and thus N availability to plants. In the coastal region along the Gulf of Bothnia such studies are scarce (Aikio et al. 2000), but comparable successional ecosystems have been studied intensively in Alaska at Glacier Bay National Park (Bormann & Sidle 1990, Chapin et al. 1994), and in the Tanana river floodplain (Klingensmith & Van Cleve 1993, Van Cleve et al. 1993, Clein & Schimel 1995, Schimel et al. 1998).

In chronosequence studies conducted in Alaskan conditions, the rate of net N mineralisation has been shown to decline with advancing succession from the poplar-alder (*Populus balsamifera* L. – *Alnus tenuifolia* Nutt.) stage

towards the mature white spruce (*Picea glauca* (Moench) Voss) stage (Klingensmith & Van Cleve 1993, Van Cleve et al. 1993). Aboveground net primary productivity of *Picea* has been shown to decrease by 50% over a 160-year, *Picea*-dominated portion of a chronosequence studied by Bormann & Sidle (1990). The changes in N availability, and hence productivity, are concluded to be related to changes in organic matter quality, through the control of microbial activity (Van Cleve & Yarie 1986, Bormann & Sidle 1990). Net N mineralisation was clearly related to significant increases in the lignin/N and C/N ratios in the organic layer, suggesting that early and mid-successional deciduous vegetation types produce litter that is less recalcitrant to decomposition in comparison to the litter of the late successional coniferous forest stages (Van Cleve et al. 1993, Van Cleve et al. 1996). Soil temperature also declined with advancing succession, but its relationship with net N mineralisation was not as clear as that between net N mineralisation and organic matter chemistry (Van Cleve et al. 1993). Plants may also affect N cycling by producing secondary compounds that directly influence microbial activity, acting as substrates, inhibitors or inducers (Van Cleve et al. 1991, Schimel et al. 1996, 1998, Pellissier & Souto 1999). In the study of Schimel et al. (1996), for instance, balsam poplar tannins were found to act as general microbial inhibitors, while low-molecular-weight phenolics functioned as substrates for microbial growth. In addition, monoterpenes have also been found to inhibit N mineralisation (White 1986) and nitrification (White 1986, Paavolainen et al. 1998) in coniferous forest soil.

Based on the C/N ratio of heterotrophic microbial cells and losses of C due to respiration, a C/N ratio of 30 has been proposed as the critical C/N value for detritus, above which heterotrophic micro-organisms are N limited and below which they are C limited (Tate 1995). From this it can be inferred that the microbes in the N-rich alder dominated stage may be relatively the most C limited, resulting in lower microbial biomass and activity in comparison to the subsequent stages of succession (Clein & Schimel 1995). During the succession a reduction in the N pool leads to N limitation of the microbial community. In the late successional spruce stages, recalcitrant C sources may also result in reduced microbial biomass and activity, and affect community composition (Flanagan & Van Cleve 1983, Mikola 1985, Bradley & Fyles 1995, Priha & Smolander 1999, Saetre et al. 1999, Hobbie et al. 2000, Priha et al. 2001).

1.7. Approach and aims of the study

The main objective of this study was to investigate the nutritional status and the physical, chemical and microbiological soil properties of coastal spruce forests in order to elucidate their relationship with the crown condition of spruce. At first, the crown condition of coastal spruce stands was assessed and compared to that of stands in other parts of southern Finland (I). Since any direct cause and effect responses between environmental factors and crown condition may be diverse and elusive to prove experimentally, the work was focused on producing information about correlative patterns between crown condition, tree nutrition and soil properties using sitewise data (I). The possible processes behind the patterns were then considered, i.e. they were inductively interpreted. It is important to bear in mind that this approach is not sufficient for proving causalities and, therefore, the interpretations should merely be taken as an introduction to reasonable theories. A similar approach was further applied in II, in which the variability in soil microbial activity was evaluated in relation to the quality and moisture regime of the organic layer, and soil types. Microbial activity in relation to crown condition was also investigated.

In the second part of the study (III, IV), I utilized the approach provided by post-glacial land-uplift, which allow the successional history of a coastal spruce ecosystem to be followed along a chronosequence. Hypothesizing that a reduction in the availability of nutrients (especially nitrogen) during forest succession contributes to the poor condition of the aged spruce crowns in the study area, I focused attention on the changes occurring in C- and N-related microbial activities in the organic layer along a primary successional transect (III, IV).

The specific objectives of the papers were:

- to investigate the variability in crown condition of coastal spruce in relation to foliar chemistry, soil type, and the mineral nutrient and moisture status of the organic layer (I)
- to study microbial activity in the organic layer of the coastal spruce stands growing on different soil types and with a different soil nutrient and moisture status (II)
- to investigate N transformations, and microbial activity and community structure in the organic layer along a primary successional transect on the land-uplift coast (III, IV)

2. Material and methods

The methods applied are described in detail in original papers I-IV.

2.1. Sites and study area

2.1.1. The surveys (I, II)

The Norway spruce stands studied in I and II were located along the Straits of the Gulf of Bothnia between Finland and Sweden (Raitio 1996; Fig. 1).

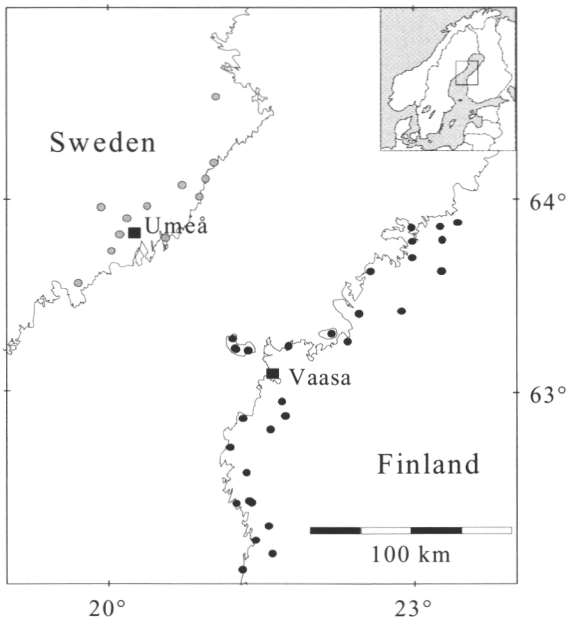

Fig. 1. The location of the sample plots studied in I (black circles) and II (black and grey circles).

Paper I focused on sites (n = 30) on the Finnish Ostrobothnian coast characterized by rapid land-uplift (Ristaniemi et al. 1998), while in II sites on both the Finnish and Swedish sides were included (n = 42). The sites were selected from the plots of the national forest inventories of Finland and Sweden, located less than 33 km from the coastline, and classified as Myrtillus (mesic) or Oxalis-Myrtillus (herb-rich) forest site types according to the Finnish forest type classification by Cajander (1949). On the Finnish side, three stand age classes were represented: 38–59 (n = 9), 60–89 (n = 11) and 90–135 (n = 10) years old. The range in altitude of these sites was 5–45 m. On the Swedish side the number of stands in age classes 60–89 and 90–135 years were 4 and 8, respectively, and the altitude of the stands varied 10–220 m.

The topography of the coastlines of the Straits of the Gulf of Bothnia is characterized by flatness and minor regional variation in altitude, especially on the Ostrobothnian (i.e. Finnish) side of the Gulf (Björklund et al. 1996, Rinkineva & Bader 1998). Glacial till deposits (De Geer moraines, drumlins and hummocky moraines) result in a special fragmented feature of the landscape in the archipelago and near the coastlines (Zilliacus 1987, Kujansuu & Niemelä 1990, Rinkineva & Bader 1998). Because of bouldering of the Vaasa granite bedrock, the soils in the northern Ostrobothnian region are generally very stony (Björklund et al. 1996). None of the studied stands were growing on acid sulphate soil, which is a type of soil that occurs sporadically in the coastal region of the Gulf of Bothnia (Merilä et al. 1996).

The annual precipitation on the Finnish side of the area ranges from 450 to 550 mm (Solantie 1987), and the effective temperature sum (threshold value of +5°C) from 1000 to 1200 d.d. (Helminen 1987).

2.1.2. The transect study (III, IV)

The transect study (III, IV) was conducted in the archipelago of Raippaluoto (Björkö and Replot) in western Finland (63°20'N, 21°15'E). The surficial deposits of the area are characterized by De Geer moraines (Zilliacus 1987). The primary successional transect was located in a nature reserve, and the impact of human activities on the development of the vegetation can be considered minor although some logging and sheep grazing might have occurred in the past. The transect comprises the following four forest sites (Fig. 2):

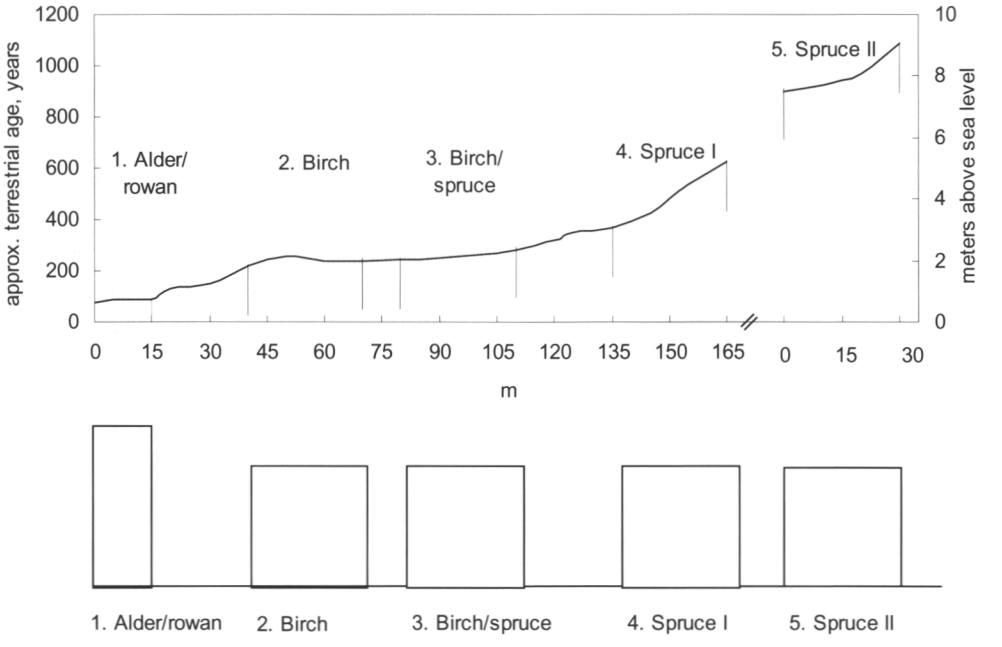

Fig. 2. The profile (above) and map (below) of the primary successional transect (III, IV). X axis refers to both figures. Terrestrial age refers to the number of years elapsed since the site rose above sea level.

(1) Alder/rowan: 70-year-old alder/rowan stand (*Alnus incana* (L.) Moench and *Sorbus aucuparia* L.)
(2) Birch: 80-year-old birch stand (mainly *Betula pubescens* Ehrh.)
(3) Birch/spruce: 75-year-old birch/spruce stand (*B. pubescens* Ehrh., *B. pendula* Roth., and *Picea abies* (L.) Karst.)
(4) Spruce I: 95-year-old spruce stand (*P. abies*)

In order to extend the age sequence, a fifth forest site was chosen 12.2 km to the south of the transect:
(5) Spruce II 130-year-old spruce stand (*P. abies*)

One sample plot (area 30 x 30 m, except for the alder/rowan site 15 x 40 m, see Fig. 2) was established in each of the forest stages. For further information see Table 1.

Table 1.
Stand characteristics, nutrient concentrations (mean ± S.E., number of trees = 10) in previous-year (c+1) needles, and thickness, loss in weight on ignition, pH($CaCl_2$), pH(H_2O), C:N ratio and total and acid ammonium acetate extractable nutrients (Halonen et al. 1983) of the organic layer of the successional forest sites studied. Measurements were carried out in 1997 or 1998.

	Alder/rowan	Birch	Birch/spruce	Spruce I	Spruce II
Stand characteristic					
Stem number ha^{-1}	1617	978	1411	589	1244
Mean diameter at breast height (cm)	13.0	23.4	20.4	26.4	22.1
Mean height (m)	7.5	14.1	14.7	16.8	16.0
Basal area ($m^2 ha^{-1}$)	9.9	20.3	26.9	19.7	26.7
Stem volume ($m^3 ha^{-1}$)	38.2	128.4	186.6	149.5	203.8
Crown defoliation of spruce (stand mean %)	–	–	18	31	32
Percentage of spruce with >5% of the needles discolored	–	–	10	20	25
Spruce c+1 needles					
N (mg g^{-1} dwt)	–	–	11.9 (0.3)	10.5 (0.3)	10.7 (0.2)
P (mg g^{-1} dwt)	–	–	2.3 (0.1)	1.9 (0.1)	1.5 (0.1)
K (mg g^{-1} dwt)	–	–	6.9 (0.2)	7.3 (0.3)	6.7 (0.3)
Ca (mg g^{-1} dwt)	–	–	4.9 (0.3)	5.0 (0.3)	5.2 (0.4)
Mg (mg g^{-1} dwt)	–	–	1.8 (0.1)	1.2 (0.1)	1.2 (0.1)
S (mg kg^{-1} dwt)	–	–	980 (30)	880 (30)	870 (30)
Cu (mg kg^{-1} dwt)	–	–	1.6 (0.1)	1.2 (0.1)	1.2 (0.1)
B (mg kg^{-1} dwt)	–	–	17.1 (0.9)	16.2 (1.2)	16.7 (1.6)
Organic layer					
Thickness (cm)	6.5	6.6	7.4	6.6	6.8
Loss in weight on ignition (OM) (%)	87.2	87.0	90.2	84.9	84.7
pH ($CaCl_2$)	3.9	3.2	3.1	3.1	3.0
pH (H_2O)	5.1	4.3	4.0	4.1	4.0
C:N ratio	15.9	20.2	21.4	31.7	37.3
Total nutrients					
N (mg g^{-1}OM)	33.0	25.7	25.1	16.0	14.3
P (mg kg^{-1}OM)	1310	1500	1690	1110	960
K (mg kg^{-1}OM)	1050	940	860	950	810
Ca (mg kg^{-1}OM)	4540	2800	1730	2980	3660
Mg (mg kg^{-1}OM)	2740	1000	650	760	720
Cu (mg kg^{-1}OM)	22.9	24.7	33.9	12.7	11.6
Extractable nutrients (mg kg^{-1}OM)					
P	200	240	160	350	200
S	150	180	200	160	140
K	840	850	750	880	650
Ca	3010	1900	1150	1980	2450
Mg	1920	760	480	550	480

2.2. Crown condition and elemental composition of the needles

The crown condition of spruce was investigated by estimating the degree of defoliation and needle discoloration (I, II; Manual on methodologies... 1989) On the Ostrobothnian (i.e. Finnish) side (I) the level of defoliation in the study area was compared with that of corresponding forest site types and stand age classes in southern Finland (demarcation line along latitude 65°). Data for this comparison were obtained from the results of the annual monitoring of forest condition carried out by the Finnish Forest Research Institute under the Pan-European Forest Condition Monitoring Programme (Forest condition... 1993).

Tree-specific samples of current (C) and previous year (C+1) needle age classes were collected from ten trees on each sample plot in December 1992 (I). The elemental concentrations of the needles were determined as described by Raitio (1991).

2.3. Soil description and sampling

In I and II the soils of the stands were described by determining the thickness of the organic layer, the soil type (FAO 1988) and the humus type (classified as mor, moder, undisturbed peat layer or disturbed peat layer). On the Finnish side, the majority (75%) of the sites were classified as stony or very stony till soils (Viro 1952). The dominant particle size was silt, fine sand and medium sand in 30%, 33% and 33% of the sites, respectively.

In the surveys (I and II), twenty-eight subsamples were collected systematically from the upper 5–7 cm of the organic layer with a stainless steel auger and combined to give one bulk sample per plot. In the transect study (III, IV), the organic layer was sampled five times (Jun –97, Jul –97, Aug –97, Sep –97 and Jul –98) by taking systematically 24 soil cores, and combining three adjacent cores to give eight subsamples per plot. In the laboratory, the samples were mixed, and litter and roots >1 mm in diameter removed.

2.4. Chemical soil properties

Total C and N were determined on a CN analyser (LECO CHN) and the organic matter content (OM) as loss in weight on ignition (485-500°C, 4 h). pH was measured after suspending a subsample in deionised water or in 0.01 $CaCl_2$ overnight (sample/liquid suspension = 1:3 v/v). Phosphorus, S,

Ca, Mg, K, Na, Al, Fe, Mn, and Cu were extracted with 1 M ammonium acetate at pH 4.65 (Halonen et al. 1983) and analysed by inductively coupled plasma atomic emission spectrophotometry (ICP, ARL 3580). Total P, K, Ca, Mg and Cu were determined by ICP after dry ashing and extracting the ash with HCl (III; Halonen et al. 1983). All the concentration data were converted to a dry organic matter basis.

2.5. Microbial activity and biomass (II, IV)

Measurement of the microbial activity of organic layer samples was conducted in constant moisture (250% of OM) and temperature conditions (+20°C) using an automated respirometer (Nordgren 1988). The samples were kept frozen prior to analysis. Basal respiration rate (BASAL), i.e. evolution of CO_2 from the sample, was first measured. Substrate-induced respiration (SIR), known to be correlated with microbial biomass (Anderson & Domsch 1978), was then determined as the respiration rate after addition of a specific substrate (glucose, N as $(NH_4)_2SO_4$ and P as KH_2PO_4). The metabolic quotient of the soil microbes (qCO_2) (Anderson & Domsch 1985a, Anderson & Domsch 1985b) was calculated as the BASAL:SIR ratio (IV), or as the relationship between BASAL and microbial biomass (II), derived from SIR values using the equation of Anderson and Domsch (1978). The additional microbial activity variables determined were Lag-time (Lag) and specific respiration increment (μCO_2), the first being estimated as the time period from substrate addition to the start of exponential growth of the microbial community, and the latter as the slope of the respiration curve during the growth.

2.6. Nitrogen transformations and microbial biomass N (III)

The estimates of net N ammonification and nitrification were determined from the change in the size of the corresponding soil inorganic-N pool over time (Hart et al. 1994). They were measured in 5-week incubation experiments *in situ* using intact soil cores, and in the laboratory on sieved, fresh organic layer samples at constant temperature (14±1°C) and moisture (250% of OM). The laboratory incubations on homogenized soil samples were intended to identify differences in substrate quality that are important for N ammonification, nitrification and immobilisation. The aim of the field incubations was to estimate the importance of environmental factors (temperature, moisture) affecting N transformations.

Total dissolved nitrogen (TDN), NH_4-N and (NO_2 + NO_3)-N concentrations in the reference (non-incubated) and incubated samples were determined from extracts (1 M KCl) on a flow injection analyser (FIA Star 5020, Tecator).

Net ammonification and nitrification were calculated by subtracting the initial NH_4-N and (NO_2 + NO_3)-N concentrations from the final (post-incubation) NH_4-N and (NO_2 + NO_3)-N concentrations, respectively. Net N mineralisation was calculated as the sum of net ammonification and net nitrification. The concentration of dissolved organic nitrogen (DON) was calculated by subtracting the NH_4-N and (NO_2 + NO_3)-N concentrations from the TDN concentration.

Gross rates of N mineralisation were estimated in the laboratory on sieved, fresh organic layer samples by the isotope-dilution technique (Hart et al. 1994). This method involves the addition of $^{15}NH_4^+$ to the sample and determination of the rate at which the atom % ^{15}N enrichment of the NH_4^+ pool decreases as microbes mineralise native soil organic ^{14}N to $^{14}NH_4^+$. It is assumed that consumptive processes do not significantly alter the ^{15}N enrichment of the NH_4^+ pool, allowing calculation of gross mineralisation from the dilution rate of $^{15}NH_4^+$ in the sample (Kirkham & Bartholomew 1954).

Soil microbial biomass N (microbial N) was determined using the fumigation-extraction method (Smolander et al. 1994). In this method, N bound in microbial cells is rendered extractable due to lysis of the chloroform-sensitive microbial cells. Microbial N is determined as from the difference in the N concentration between fumigated and unfumigated samples.

2.7. Microbial community structure (IV)

The structure of the microbial communities was estimated by determining the phospholipid fatty acid (PLFA) composition of the cell membranes. PLFA analysis was considered advantageous because it is a quick, quantitative method and does not require isolation of the microbes from the soil substrate (Balkwill et al. 1988, Frostegård et al. 1991). The total amount of PLFAs can also be used as an indicator of the living microbial biomass (Balkwill et al. 1988, Frostegård et al. 1991). Moreover, the relative amounts of PLFAs considered to be primarily of bacterial or fungal origin provide measures of the bacterial and fungal biomass (Frostegård & Bååth 1996). The PLFA composition also allows more detailed interpretations of microbial community structure, even though the indicative value of most of the single PLFAs is not clear. For example, the relative amount of PLFA16:1w5 has been reported to be higher in soil containing arbuscular mycorrhizal fungi (Olsson et al. 1995), and the methyl group in the tenth carbon atom from the carboxyl end of the chain has been found to be characteristic of actinomycetes (Kroppenstedt 1985).

2.8. Statistics and ordinations

The Pearson (r) or the Spearman rank correlations (r_S) were calculated in order to evaluate the covariation between the variables studied (I-IV). The analysis of variance and nonparametric Kruskal-Wallis test were used to distinguish differences between the groups. Pairwise differences were tested with Tukey's test and, in case where the equality of variances between the groups was not met, pairwise comparisons were made for mean ranks (rejection level 0.05).

Because the transect studies (III, IV) were conducted on a single transect and the successional stages were thus not replicated, statistical comparisons can be made to distinguish statistically significant differences between sites but not between the successional stages. Differences in the variables between the plots and between the 1997 incubations were tested with repeated measures analyses of a general linear model. When the assumption of sphericity was not violated according to Mauchly's test (Crowder & Hand 1990), contrasts were used to test the differences among variables between subsequent incubations (SPSS® version 9.0.1).

In III the linear mixed model analysis (PROC MIXED procedure SAS 6.12 software package) was used to investigate the degree to which certain properties of the organic layer accounted for differences in net N mineralisation between the forest sites, and between the incubations in the laboratory (alder/rowan site excluded). The incubations were treated as repeated measures.

In I, principal component analysis (PCA), based on the correlation matrix (Jongman et al. 1987), was applied to sum up the variation of intercorrelated original variables into one principal component. The input variables in PCA were: N in current needles, B in previous-year needles, and the total N and extractable S concentrations, pH, and moisture content of the organic layer at the time of sampling. The site scores along the first PCA axis were interpreted to reflect increasing fertility and decreasing moisture and named as the site fertility index.

In II, the relationships between variables depicting microbial activity and site and organic layer characteristics layer characteristics were investigated by redundancy analysis (RDA), which is a multivariate linear method and a canonical form of PCA, designed to detect the main relationships between two sets of variables (Jongman et al. 1987). RDA analyses were performed on a correlation matrix using CANOCO version 3.10 (ter Braak 1990). Variables describing soil microbial activity, i.e. BASAL, SIR, Lag, μCO_2 and qCO_2, were entered as dependent variables ("species") in the program, and organic layer chemistry and site variables were entered as independent (environmental) variables.

In IV the PLFA data were ordered by global non-metric multidimensional scaling (NMS) using PC-ORD software 4.14 (McCune & Mefford 1999). Prior to NMS, the mole percentages of the PLFA values were double-square root transformed ($y^{0.25}$) in order to down-weight the influence of the very abundant PLFAs. Sørensen (Bray & Curtis) distance was applied as a measure of dissimilarity in microbial community structure between the samples. The C/N ratio and pH in the organic layer, BactPLFA, TotPLFA and the FungPLFA/BactPLFA ratio were given as vectors in the ordination graph, the direction of each arrow indicating the direction of the gradient and the length indicating the strength of correlation. The final configuration was rotated by pH.

3. Results

3.1. The surveys (I, II)

The most common soil types on the sites studied on the Finnish coast were carbic podzols and dystric gleysols, and crown condition was found to be the worst in old stands growing on these soil types (Fig. 4 in I). Defoliation and discoloration correlated positively with stand age (Table 2 in I). The spruce stands older than 60 years were more defoliated in coastal Ostrobothnia than in other parts of southern Finland (Fig. 3 in I).

Old stands in which the spruces were highly defoliated and discoloured had low needle N and Cu concentrations. Boron needle concentrations were highest in these stands. Total N and extractable S concentrations in the organic layer decreased with increasing stand age, defoliation and discoloration.

Principal component analysis (PCA) was applied in order to sum up the variation in the intercorrelated needle chemistry and organic layer variables. The input variables were N in current needles, B in previous-year needles, and total N, extractable S, pH(H_2O) and the moisture content in the organic layer at the time of sampling (Table 3 in I). The site scores along the first PCA axis were used as the site fertility index (increasing fertility, decreasing soil moisture gradient). Consistently with the input variables, this index showed significant (negative) correlation with crown defoliation, crown discoloration and stand age. Stand age-adjusted partial correlation coefficients of the elemental needle concentrations and soil properties with crown defoliation and discoloration were insignificant, apart from the correlation between site fertility index and crown discoloration ($r = -0.41$, $p = 0.03$, $df = 27$).

The organic layer of the ferric podzols had higher BASAL and SIR than the carbic podzols and higher SIR than the gleysols (Table 4 in II). BASAL and SIR were positively related to organic layer fertility factors such as pH and extractable K, Mn and P, but negatively associated with the organic matter

content of the organic layer (Fig. 2 in II). A short Lag was associated with organic layer fertility and a long Lag with crown defoliation. μCO_2 was lowest at sites with a high field moisture and discoloured crowns. qCO_2 was situated near the centre of the ordination space, thus indicating poor, if any, correlation with the environmental variables. No correlations were found between microbial activity variables and stand age.

3.2. The transect study (III, IV)

Along the primary successional transect (alder/rowan, birch, birch/spruce, spruce I, spruce II), the C/N ratio in the organic layer increased from 16 to 37 and the pH(H_2O) decreased from 5.1 to 4.0 (III). Concurrently, net N mineralisation decreased substantially (III). The alder/rowan site was the only site to show net nitrification.

Net N mineralisation per unit area summed from the four field incubations (3 Jun – 21 Oct 1997) was 7.4, 7.4, 7.0, 3.6 and 2.8 g m^{-2} in the alder/rowan, birch, birch/spruce, spruce I and spruce II sites, respectively. It was significantly lower in spruce sites I and II than in the other sites.

The average N mineralisation coefficient (net N mineralisation during Jul –98 laboratory incubation: total N) was 0.008, 0.009, 0.010, 0.005 and 0.005, in the alder/rowan, birch, birch/spruce, spruce I and spruce II sites, respectively. The average N mineralisation coefficient thus tended to show lower values in the spruce sites, but the differences between the sites were only tentative.

BASAL and SIR remained mainly stable although, at the time of the most favourable temperature and moisture conditions (BASAL Jul –97; SIR Jul –97 and Jul –98), they tended to increase along the transect from the alder/rowan site to spruce I, and decreased again in spruce II (IV). qCO_2 showed no consistent trend along the transect.

In contrast to the clear decline in the net N mineralisation rate along the transect, the average gross N mineralisation rate showed a slight increasing trend, although the differences between the forest sites were not significant (Fig. 6a in III).

Microbial biomass N was the lowest at the alder/rowan site, and differed significantly from that in spruce I (Fig. 6b in III). The proportion of microbial biomass N out of total N in the organic layer was 2.4, 4.1, 4.1, 6.7 and 5.7% in the alder/rowan, birch, birch/spruce, spruce I and spruce II sites, respectively. The response of microbial N to the C/N ratio was concavely curvilinear, with the lowest microbial biomass N at the lowest (alder/rowan) and the highest (spruce II) C/N ratios.

The organic layer of the birch site had the highest total PLFA and bacterial PLFA concentrations, while that of both spruce sites had the lowest (IV). The ordination configuration of the PLFA data in non-metric multidimensional scaling was clearly related to the C/N ratio and pH, and separated the forest sites relatively well (Fig. 1a in IV). It was possible, on the basis of the similarities in the variation pattern along the transect, to divide the PLFAs into 6 groups (Fig. 1b and Table 3 in IV). The PLFAs of Group 1(16:1w5, cy17:0, 18:1, 18:1w7, 10Me18:0, 19:1a) were relatively more abundant in the alder/rowan site in comparison to the other sites. Group 2 (i14:0, a15:0, i17:0, br18:0) also showed the highest relative amounts in the alder/rowan site and, in addition, gradually decreased along the transect. Group 3 consisted of seven PLFAs (i16:0, i16:1, 10Me16:0, 10Me17:0, 17:0, br17:0, 18:0), which were the most abundant either in the birch site or in the birch/spruce site, and three of them (i16:0, i16:1 and 10Me17:0) were present in minimum amounts in spruce sites I and II. Group 4 (i15:0, cy19:0, c16:0) was characterized by a relatively low abundance in the alder/rowan site compared to the other sites. The feature common to the PLFAs of Group 5 (13 PLFAs, see Table 3 in IV) was that each of them showed the highest relative abundance in either of the spruce sites. Group 6 included PLFAs showing stable or inconsistently variable amounts along the transect (Table 3 in IV).

4. Discussion

4.1. Crown condition vs. site conditions

Study (I) showed, as in several earlier studies (Thomsen & Nellemann 1994, Müller-Edzards et al. 1997, Lindgren et al. 2000), that crown defoliation and discoloration are clearly associated with stand age. However, the fact that the spruce stands older than 60 years were more defoliated in the studied coastal sites than in other parts of southern Finland (I) indicates that the crown condition of coastal spruce is affected by factors other than only the aging of the stand. The old stands with the worst crown condition were growing on infertile sites on periodically waterlogged soil types (dystric gleysols and carbic podzols; I). This result indicates that changes in the microclimate and nutrient cycling during the succession of spruce forests leads to paludification (cf. Sirén 1955) and to a deterioration in site fertility (cf. Ranger & Nys 1994), resulting in impaired crown condition. Changes occurring in the properties of the organic layer and in the ground vegetation along the primary succession transect support this interpretation (III). The rather drastic conclusion of 'suicide succession' of the coastal spruce stands

should, however, be avoided. The result may be biased by the fact that old stands growing on infertile, paludified sites are not very attractive subjects of felling and regeneration activities, and are therefore readily left uncut. In actual fact, such sites became selected in this study as representatives of old coastal spruce stands. Our results are consistent with the results obtained in more extensive surveys in Finland (Lindgren et al. 2000) and in Norway (Thomsen & Nellemann 1994), according to which the defoliation degree of spruce increases with decreasing site fertility, in addition to the evident relationship between defoliation and stand age. The results suggest that the poor crown condition of the coastal spruce stands is related to natural soil factors such as periodic water-logging and low fertility. These factors are likely to affect the condition of spruce in the region, since infertile, stony and paludified sites are more common on the western coast than in the inland parts at the same latitudes of Finland (Karlsson 1996). Fertile and productive spruce sites do, however, also exist on the Ostrobothnian coast, especially near the coastline where the soil nitrogen capital has been amended by nitrogen-fixing alder species (*Alnus incana* (L.) Moench and *Alnus glutinosa* (L.) Gaertner) (Erikslund 1997).

Crown discoloration in the studied stands appeared in most cases as yellowing of the older needles in the upper part of the crown, the younger needles in the topmost part of the crown remaining symptom-free (Salemaa et al. 1996). Even after adjustment for stand age, this symptom showed significant negative correlation with the site fertility index. The site fertility index was created from the site scores of the first axis of principal component analysis ('increasing fertility, decreasing moisture'), in which needle N and B concentrations and total N, extractable S, pH and moisture content of the organic layer were used as input variables. This result indicates that soil properties play a significant role in crown condition.

Stoniness has often been mentioned as a factor having a significant influence on the growing conditions of forests along the land-uplift coast in western Finland (Appelroth 1948, Kuusela 1977). Most of the sites sampled on the Finnish coast were classified as stony or very stony (I), but there was, however, no significant covariation between the stoniness index and crown condition, needle chemistry or properties of the organic layer (data not shown). Karlsson (2000) presented height curves for coastal Scots pine and Norway spruce in western Finland, and found that stony sites showed a growth pattern of stronger stagnation over age than stone-free sites, but only for pine. He concluded that the poor rooting conditions on stony sites increase the susceptibility of trees to climatic factors such as strong winds and summer drought, which are characteristic of the weather conditions prevailing in the coastal area (Heino 1987, Solantie 1987). In the spruce data, part of which was collected from

the same sites as those studied in I and II, Karlsson (2000) found a stronger stagnation of growth over age at sites with a thick humus layer compared to those sites with a thin humus layer. This probably reflected the slower rate of nutrient cycling in paludified sites with a thick humus layer.

Since acid sulphate soils occur sporadically in the coastal regions of the Gulf of Bothnia, excessive acidity is another factor that may have an influence on the growing conditions in the study area. These soils, formed during the early stages of the Litorina Sea, have been estimated to cover over 2000 km^2 of land in Finland (Palko & Lakso 1991). However, there were no indications to suggest that the forest sites studied included acid sulphate soils. For example, the sulphur concentrations in mineral soil layers of the study sites did not correspond the high levels reported for the soil horizons of acid sulphate soils (0.2–3.7% dw.; reviewed by Palko 1994). In fact, the sulphur concentrations in the mineral soil layers of the study sites were very similar to those reported for spruce forest soils in southern Finland (Merilä et al., 1996, Tamminen, unpublished results).

4.2. Possible effects of waterlogging and low soil temperature

Waterlogged, anaerobic soil conditions impair the nutrient cycling of forest ecosystems in several ways. Such conditions impede the growth and respiration of the roots and, consequently, the uptake of water and mineral nutrients (Marschner 1995), especially NH_4^+ (Lévy 1981), which is the dominant form of mineral N in acidic, sporadically waterlogged soil conditions (Smolander et al. 1995, Stark & Hart 1997, III). The nitrogen deficiency in the spruce stands studied here might thus be due to impaired N uptake. One factor which probably further hinders nutrient and water uptake in the study area is the low soil temperature or even soil frost at the beginning of the growing season. The key climatological factors determining the depth of soil frost are the winter index (sum of the mean daily air temperatures below 0°C) and thickness of the insulating snow cover (Huttunen & Soveri 1993). The Ostrobothnian region is characterized by low winter precipitation: the long-term average for the winter maximum snow cover depth is circa 40 cm, while, for example, the snow cover at the same latitudes in eastern Finland is twice as thick (Solantie 1987). Due to the thin snow cover, the soil freezes down to a greater depth and persists for a longer period on the western coast compared to inland areas, even though the winter index is lower in western Finland than in the eastern part of the country (Huttunen & Soveri 1993). The efficient interception of snowfall in a closed spruce canopy further reduces

the thickness of the snow cover and delays soil warming in the spring (Mustonen 1966, Wulff 1996, Merilä 2000). The spruce stands in the coastal Ostrobothnian region are thus susceptible to desiccation injury during sunny days in early spring when the leaf temperature rises above freezing and transpiration increases, but water cannot be absorbed through the roots fast enough to replace transpirational losses (Kozlowski et al. 1991).

Waterlogging and low temperatures also decrease soil microbial activities (Mikola 1960, Van Cleve et al. 1990, Blume et al. 1991). These environmental conditions, together with the acidic soil and recalcitrant needle litter, evidently result in the immobilisation of essential nutrients, especially N, in the forest floor (Flanagan & Van Cleve 1983, Bormann & Sidle 1990). This conclusion is supported by the results of II showing a lower basal respiration (BASAL) in the organic layer of carbic podzols, and a lower microbial biomass (SIR) in both the carbic podzols and dystric gleysols, compared to the BASAL and SIR in the organic layer of ferric podzols. The ratio of BASAL and SIR (qCO_2), i.e. the index of carbon use efficiency (Wardle & Ghani 1995, Wardle et al. 2001), showed no significant differences between the soil types, nor any covariation with the properties of the organic layer. The factors contributing to the value of this ratio thus remained unclear (see also chapter 4.5.4). Although no significant differences between the soil types were apparent in μCO_2, it seemed to respond to excess soil moisture. The lowest values were found in samples with the highest field moisture content, although the actual respiration measurements were conducted in ideal moisture conditions (250% OM). This may also be a consequence of the fact that, in wet conditions, the microbial community is to some extent adapted to anaerobic conditions and, consequently, not as much CO_2 is released as in well aerated conditions.

The results of the transect study (III, IV) offered some support for the hypothesis that excess soil moisture and consequent paludification result in reduced nutrient availability, which further contributes to the reduced crown condition observed in the study area. The old paludified spruce II site repeatedly had the lowest net N mineralisation rates along the primary successional transect, and the N mineralisation coefficient (net N mineralisation during Jul –98 laboratory incubation : total N in the organic layer) tentatively declined along the transect (III; see also chapter 4.5.). Since gross mineralisation concurrently remained relatively stable, the decrease in net N mineralisation was interpreted to be primarily due to the increase in microbial immobilisation.

4.3. Microbial activity and biomass vs. nutrient concentrations in the organic layer

BASAL and SIR correlated positively and Lag correlated negatively with the concentration of extractable K of the organic layer (II). Potassium is characterized by a high mobility in plants; it is not metabolised, and forms only weak complexes (Marschner 1995). Consequently, potassium is rapidly released from plant detritus, e.g. from needle litter (Staaf & Berg 1982, Bockheim et al. 1991), which also indicates that K does not limit microbial growth. The correlation between microbial activity and K may reflect the proportion of mineral soil material in the organic layer, which in this material can be assumed to increase with increasing fertility. Furthermore, Palmborg (1997) found a similar positive correlation between K concentration and respiration rate in the mor layer, and proposed that this relationship might reflect the abundance of bilberry (*Vaccinium myrtillus* L.), the leaves of which are rich in potassium and are also easily decomposed (Johansson 1993). Microbial activity and biomass were also rather highly correlated with Mn. Mn has been shown to be essential for the activity of the lignolytic enzymes (Archibald & Roy 1992, Perez & Jeffries 1992), and decomposition activity may even be regulated by the availability of Mn (Berg et al. 1995).

4.4. Crown condition vs. needle elements

Of all the measured mineral elements in the needles, B had the strongest relationship with crown condition, being positively correlated with crown defoliation (I). Similar correlation has been found in another Finnish study covering 43 Norway spruce stands in southern Finland (Lindgren et al. 2000). Elevated foliar B concentrations have also been reported earlier in studies on reduced living crowns of Scots pine caused by browsing moose (Löyttyniemi 1985), Scleroderris canker (*Gremmeniella abietina* (Lagerb.) Morelet) infections and manual pruning (Nuorteva & Kurkela 1993, 1998). The distribution of B in plants is primarily governed by the transpiration stream: the regulation of B uptake and translocation is rather limited compared to that of other mineral nutrients (Marschner 1995). One possible explanation for the elevated B concentrations might thus be that B simply accumulates in remaining needles of a defoliated crown, but further studies would be necessary in order to test this hypothesis.

The N and Cu concentrations in the needles were negatively correlated with crown defoliation and discoloration (I). Needle N and Cu concentrations were also clearly positively correlated with each other. Similar covariations

were also found in the study of Lindgren et al. (2000). Our results are consistent with those of van den Burg (1983), who concluded that, at least under conditions of low N availability, N seems to stimulate Cu uptake. In a nutrient optimisation experiment in young Norway spruce stands in northern Sweden, the Cu concentrations and Cu/N ratios in the needles were found to remain relatively constant over time and between treatments, despite large differences in biomass production (Linder 1995). This observation, which indicates that Cu is taken up in relation to the demand rather than to the supply, fits well with our results. However, in comparison with the optimum value of 0.02 (Linder 1995), the Cu/N ratios found in our study were relatively low, varying in the range of 0.0062 – 0.0173. In addition to low concentrations in the parent material, Cu availability may be reduced as a result of the complexation of Cu with organic compounds (Stevenson & Fitch 1981). Moreover, a low temperature has been shown to affect strongly the desorption of Cu (McLaren et al. 1990). Low needle Cu in spruce stands has been also observed in other parts of Finland, both on peatlands (Silfverberg 1980) and on mineral soils (Raitio 1994). However, the visible Cu deficiency symptoms described by van Goor & Henkens (1966) and van Goor (1968) have so far not been reported in Finland.

4.5. Soil microbial dynamics along the primary successional transect

4.5.1. Net N mineralisation

Net N mineralisation decreased along the primary succession transect (alder/rowan, birch, birch/spruce, spruce I and II; III) in both the field and laboratory incubations. These results confirm the results obtained in successional floodplain soils along the Tanana River, in interior Alaska. In this area, net ammonification and nitrification were at their highest in the middle successional poplar-alder forest floor, while in the late successional white spruce forests they were at their lowest or even undetectable level (Klingensmith & Van Cleve 1993). A decrease in net ammonification and nitrification during the course of succession has also been reported in a number of other studies, as reviewed by Robertson (1982). The transect studied here – alder/rowan, birch, birch/spruce, spruce I and spruce II – is clearly a sequence ranging from a N-rich ecosystem characterised by easily degradable litter and low canopy interception, to a N-poor ecosystem with highly recalcitrant litter and high canopy interception. Our estimates for the net N mineralisation on an areal basis (2.8 – 7.4 g m^{-2}) are fairly consistent with

those reported in Norway spruce stands in Sweden and Denmark (1.7-6.8 g m^{-2} yr^{-1} in the LFH layer; Persson & Wirén (1995)).

The N mineralisation coefficient (Weier & MacRae 1993), i.e. net N mineralisation as a proportion of the total N concentration of the organic layer, tentatively declined in the spruce sites compared to the sites representing earlier successional stages. This is assumed to be an indication of the more recalcitrant nature of soil organic matter, generally expressed as a higher lignin content and C/N ratio (Berg 1986) in late successional spruce forests than in the preceding deciduous stands (Pastor et al. 1987, Priha & Smolander 1999, Côte et al. 2000). Initial concentrations of NH_4-N and DON in the samples seemed to predict well the actual net N mineralisation rate, since these variables accounted for the differences in net N mineralisation between the forest sites, as well as between the incubation periods (alder/rowan site excluded). The initial DON concentration evidently depicts the easily mineralisable N pool in the samples and, together with NH_4-N, also indicates the recent activity of soil microbes.

4.5.2. Net nitrification in the alder/rowan site

Net nitrification only occurred in the alder/rowan site. This result is consistent with the findings of Van Cleve et al. (1993) and Hart & Gunther (1989). The latter authors found net nitrification in the soil only in the alder site when four different subarctic vegetation types (alder, dry tundra, moist tundra and white spruce sites) were compared. However, a lack of net nitrification does not necessarily indicate the absence of nitrifiers, because the nitrate formed may be rapidly immobilised by soil micro-organisms (Stark & Hart 1997, Stottlemyer & Toczydlowski 1999). Dinitrogen-fixing alder influences mineral soil N transformations both by increasing the size of the total N pool in the soil, and by supplying higher quality litter inputs to the forest floor than non-dinitrogen-fixing plants (Hart & Gunther 1989). In our study the organic layer of the alder/rowan site differed from that of later successional sites in having a higher pH and greater N availability. Plant secondary compounds may also have played a role in the cessation of net nitrification after the transition from a dominance of alder and rowan to birch, and further to spruce. In Alaskan river floodplains, for instance, the rapid decrease in N_2 fixation and nitrification during the transition from alder to balsam poplar has been attributed to the effects of secondary compounds in balsam poplar on microbial activity (Schimel et al. 1996, Schimel et al. 1998, Fierer et al. 2001).

Net nitrification in the alder/rowan site was found to correlate positively with pH($CaCl_2$) in the range of 3.32–4.84, which is in accordance with the results of other studies (Smolander et al. 1998, Ste-Marie & Paré 1999). Net

nitrification was also positively correlated with the initial concentrations of TDN, mineral N and (NO_2 + NO_3)-N, but not with that of NH_4-N. Although NH_4-N is the substrate for autotrophic nitrifying bacteria, the initial concentration of (NO_2 + NO_3)-N, which is the net product of recent nitrification activity, showed a better correlation with the rate of net nitrification than the initial concentration of NH_4-N. This indicates that, in conditions favourable for nitrification activity, the NH_4-N produced in ammonification is rapidly consumed by nitrifying bacteria.

4.5.3. Gross N mineralisation and microbial biomass N

In contrast to decreasing net N mineralisation rates, the gross mineralisation of N showed a tentative increase along the transect, although the differences between the forest sites were non-significant. Similarly, in a study on net and gross N mineralisation below birch (*Betula papyrifera* Marsh.), spruce (*Picea glauca* (Moench) A. Voss) and alder (*Alnus incana* (L.) Moench) in Isle Royale, Michigan, the alder forest showed the highest net N mineralisation rate, but gross mineralisation was the highest beneath spruce and birch (Stottlemyer & Toczydlowski 1999). The authors concluded that the higher net N mineralisation rates beneath alder in comparison to birch and spruce resulted from lower microbial immobilisation rather than greater gross N mineralisation. In our study, microbial biomass N was not followed during the incubation, but the increasing microbial biomass N (measured in Jul-98) along the transect from alder/rowan to spruce I, in part supports this interpretation. However, greater immobilisation of N in the late successional spruce sites is probably an inadequate explanation for the stable or even increasing gross N mineralisation rate and decreasing net N mineralisation rate along the transect, since we cannot assume that the microbial N pool will continuously increase. This contradiction in the results could be due to the $^{15}NH_4^+$ pool dilution method used, as discussed in the recent paper of Fierer et al. (2001). The proportion of microbial biomass N out of total N in the organic layer increased along the transect, and hence the rate of gross N mineralisation per unit of microbial biomass N remained relatively stable (data not shown). Thus, the gross mineralisation rates observed would appear plausible if pool dilution measured the microbial cycling and recycling of small pools of highly labile, N-rich compounds rather than the overall decomposition of soil organic matter and microbial growth, as suggested by Fierer et al. (2001). Further, it may be hypothesized that, in the late successional spruce sites, the higher proportion of N in the microbial pool, compared to the earlier sites, will be transformed into the more stabile N pool, i.e. to humic substances, resulting in a decreasing net N mineralisation along the transect.

Microbial biomass N tended to increase along the transect from the alder/rowan site to spruce I, but was lower again in spruce II. Correspondingly, since the C/N ratio increased along the transect, the response of microbial biomass N to the C/N ratio was concavely curvilinear rather than linear. This may indicate that the microbes in the N-rich alder/rowan site are relatively C limited, as reported earlier for an early successional alder stage by Clein & Schimel (1995). During the succession, increasing C availability creates conditions in which the microbial biomass can increase, but a reducing N pool leads to N limitation of the microbial community (Ohtonen et al. 1992, Aikio et al. 2000). In the spruce II site, microbial growth may again become limited by factors other than nitrogen, e.g. by the presence of more recalcitrant C sources.

4.5.4. Microbial respiration, biomass and the carbon use efficiency

Along the primary successional transect studied (alder/rowan, birch, birch/spruce, spruce I, spruce II) we hypothesized a concavely curvilinear response of basal respiration (BASAL) and microbial biomass (SIR) to changing organic matter quality, which was primarily indicated by an increasing C/N ratio and decreasing pH and net N mineralisation (III).

In contrast to our hypothesis, BASAL and SIR were relatively stable along the transect. This result can be interpreted to indicate that the decomposition of aged soil organic matter occurs over a wide range of substrates at a relatively stable rate, or that the labile C pool was able to maintain microbial activity irrespective of the size and decomposability of the recalcitrant C pool. Similarly, BASAL and SIR remained unchanged along a fertility gradient studied by Pennanen et al. (1999). However, Nohrstedt (1985), who investigated microbial activity in forest floors using bulked samples of three samplings during one growing season, found a curvilinear response between respiration and the C/N ratio in the organic layer, and concluded that optimum conditions for decomposition were within the C/N ratio range 20–30. In our study, the samples taken during the most favourable temperature and moisture conditions in the field (BASAL in Jul –97 and BASAL and SIR in Jul –98) tended to show this pattern, i.e. BASAL and SIR increased slightly along the transect from alder/rowan to spruce I, but were again lower in spruce II, and thus partly supported our hypothesis. Consistent support for the hypothesis that N limitation or more recalcitrant C sources would reduce microbial biomass and activity in the late successional spruce site was not, however, obtained.

In the alder/rowan site, microbial activity may be affected by the influence of a high N concentration on the degradation or degradability of lignified organic substances. N-rich litter degrades relatively rapidly in the early stages of decomposition but, during the later stages, negative correlation has been repeatedly reported between the nitrogen concentration and the rate of loss of lignin mass (Berg et al. 1982, Berg & Wessen 1984, McClaugherty & Berg 1987, Berg & Ekbohm 1991). A similar relationship has also been found between the basal respiration and N concentration in the humus layer (Berg & Matzner 1997, Persson et al. 2000). Accumulation of soil organic matter in the early stages of primary succession is an important process which, by increasing the water-holding and cation exchange capacities, facilitates the establishment of later successional species. The finding that N-rich litter has a larger recalcitrant fraction than N-poor litter and, consequently, results in higher accumulation of soil organic matter in relation to the amount of litterfall (Berg et al. 2001), thus appears to be very appropriate from the successional point of view.

BASAL and SIR in spruce I site were occasionally surprisingly high. The highest rates were actually observed on stony, and therefore dry, infertile patches with a thin, poorly decomposed organic layer. These patches obviously had a high density of fine roots and associated ectomycorrhizal hyphae. It can be assumed that the mycorrhizal hyphae, still present in the sample after sieving, continued to respire after excision from their host and, together with the root exudates remaining in the sample, also provided a source of substrates for decomposing microbes, resulting in the relatively high BASAL, SIR and FungPLFA measured in spruce I site. Moreover, the high microbial activity and biomass measured in this site may have reflected the favourable temperature and moisture conditions in the field, since poorly decomposed organic material evidently contains easily decomposable C sources for soil microbes. The presence of poorly decomposed organic material may also partly explain the highest FungPLFA concentration in spruce I, because fungal communities have been found to play a dominant role in litter breakdown in the early stages of decomposition (Dilly et al. 2001).

qCO_2 showed no significant differences between the successional sites in three of the five samplings, and the birch/spruce (Jul –97) and birch and birch/spruce (Aug –97) in the other two samplings tended to show higher qCO_2 than the other sites. In a recent paper, Vance & Chapin (2001) suggested that differences in qCO_2 between forest ecosystems may reflect several kind of disparities, such as differences in the proportion of inactive microbial biomass, in the degree of substrate limitation of microbial activity or in the metabolic rates, turnover, and growth efficiency of different microbial

functional groups. In our study (I) these factors seemed to counteract each other and few, if any, differences were found between the sites. While qCO_2 undoubtedly indicates microbial efficiency, this quotient appears to be too unspecific to reflect ecosystem development (Wardle & Ghani 1995).

4.5.5. Microbial community structure

As revealed by NMS ordination of the PLFA data, the microbial community structure showed relatively clear differences along the transect, and was closely related to the C/N ratio and pH of the soil. It was possible, on the basis of the similarities in the variation pattern along this transect, to divide the PLFAs into 6 groups, even though the indicative value of most of the single PLFAs is not clear. The most distinctive group in NMS ordination was formed by the samples from the alder/rowan site. The amount of PLFA 16:1w5, which has been reported to be present in higher amounts in soil containing arbuscular mycorrhizal fungi (Olsson et al. 1995), was at its maximum in this site. The result is consistent with the fact that the understorey vegetation in the alder/rowan site was dominated by grasses and herbs (III), which generally form this type of mycorrhizal association.

5. Concluding remarks

In summary, the results suggest that the poor crown condition of the coastal spruce stands is related to natural soil factors such as periodic water-logging and low fertility. These factors are likely to affect the condition of spruce in the region, since infertile, stony and paludified sites are more common on the western coast than in the inland parts at the same latitudes in Finland. Climatic factors such as persistent soil frost caused by the thin snow cover may be an additional stress factor that impairs water and mineral nutrient uptake at the beginning of the growing season.

The case study on the dynamics of C- and N-related soil microbial activities along a successional transect provided evidence of distinctive changes in organic matter quality and decreasing mineral N availability during forest succession. Low N availability may contribute to the poor crown condition and growth of the aged Norway spruce forests on the land-uplift coast in western Finland, although the ability of mycorrhizal roots to utilise organic forms of nitrogen may, in part, compensate for the reduced availability of mineral N. Moreover, it should be borne in mind that N mineralisation rates obtained in incubation experiments are merely indications of the actual N supply, and should not be taken as accurate determinations. Microbial biomass and respiration were relatively stable among the successional forest sites, in

spite of the clear differences in the structure of the microbial community along the transect. During the most favourable temperature and moisture conditions in the field, however, these variables tended to increase along the transect from alder/rowan to spruce I and decreased in spruce II site, suggesting that seasonal factors may account for the observed variation. The contribution of abundant mycorrhizal hyphae to microbial respiration and biomass, especially in the spruce sites, cannot be ruled out either.

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Corrections

- Paper I, p. 415, column 1, line 1. LECO analyser used was CHN-600, not TGA-500.
- Paper I, p. 416, column 2, line 1. 4a-c, not 2a-c.
- Paper II, p. 362, column 1, line 4. LECO analyser used was CHN-600, not TGA-500.
- Paper III, p. 378, Table 3. Letter b, denoting the significant differences between the groups, is missing from the initial concentration of NH_4-N (mg kg^{-1} OM) in spruce I site in Jun -97.
- Paper III, p. 382, column 1, line 4-5. The reference Persson et al. 1995 should be replaced with Persson, T. & Wiren, A. 1995. Nitrogen mineralization and potential nitrification at different depths in acid forest soils. Plant and Soil 168-169:55-65.

Paper I

Reprinted from Scandinavian Journal of Forest Research, Vol. 13, Merilä, P., Lindgren, M., Raitio, H. & Salemaa, M. Relationships between crown condition, tree nutrition and soil properties in the coastal *Picea abies* forests (Western Finland), 413–420, copyright 1998, with permission from Taylor & Francis.

I

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Relationships Between Crown Condition, Tree Nutrition and Soil Properties in the Coastal *Picea abies* Forests (Western Finland)

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The state of health of 30 Norway spruce (*Picea abies* L. Karst.) stands located on the uplifting coast of western Finland was examined in 1992. Relationships between crown condition, elemental concentrations of the needles, organic layer properties and soil type were studied using sitewise data. The site scores along the first axis of the Principal Component Analysis for certain needle and organic layer variables were used as the site fertility index. The spruces in the study area showed a higher level of defoliation and this occurred in younger stands than elsewhere in southern Finland. Defoliation and discoloration correlated positively with stand age, and negatively with the site fertility index. Low N and Cu concentrations of the needles were found in older stands where spruces were highly defoliated and discolored. Boron concentrations of the needles were highest in these stands. Total N of organic layer and extractable S concentrations were negatively correlated with stand age, defoliation and discoloration. The results suggest that poor crown condition of the coastal spruce stands is related to natural soil factors such as periodic water-logging and low fertility. Long-lasting soil frost as a consequence of thin snow cover might be an additional stress factor impeding water and mineral nutrient uptake in the beginning of the growing season. *Key words: defoliation, discoloration, forest condition, needle analysis, Picea abies, soil fertility.*

INTRODUCTION

The poor condition of Norway spruce (*Picea abies* (L.) Karst.) forests located in the coastal area of the Gulf of Bothnia, western Finland, has been causing concern for several decades (e.g. Appelroth 1948). A special characteristic of these coastlines is the land uplift (8–9 mm a^{-1}, Mäkinen et al. 1986) that continuously exposes new land to soil-formation processes (Starr 1991) and provides surfaces for colonization by plant communities (e.g. Palomäki 1963). The forest vegetation succession on stony, fine-grained till soils starts from alder-dominated (*Alnus incana* (L.) Moench) deciduous shoreline vegetation and ends in almost pure Norway spruce forests (Appelroth 1948).

Large-scale monitoring of forest condition in Europe is based on the assessment of foliage loss (defoliation) and discoloration (Anon. 1989). The main objective of these surveys has been to assess the effect of air pollutants on tree vitality. However, it is generally agreed, that defoliation and discoloration are unspecific indicators of tree vitality. Both may be strongly affected by natural factors such as tree age, climate and soil conditions. Many authors have emphasized the additive effects of natural and anthropogenic factors (e.g. Zöttl & Hüttl 1986, Schulze 1989, Salemaa et al. 1991, Chappelka & Freer-Smith 1995). In the case of coastal spruce forests, natural factors can be assumed to be the primary cause of poor crown condition, since their poor condition was already noted when the emissions of air pollutants were low (Appelroth 1948).

In this study the crown condition of spruce in the coastal area was described and compared to that in southern Finland. The variability in crown condition of coastal spruce was evaluated in relation to foliar chemistry, soil type, and the mineral nutrient and moisture status of the organic layer.

MATERIAL AND METHODS

The studied Norway spruce stands ($n = 30$) are located in the coastal area of the county of Vaasa, Finland (Fig. 1) and classified as Myrtillus (mesic) or Oxalis-Myrtillus (herb-rich) forest site types accord-

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ing to Cajander (1949). Three stand age classes are represented: 38–59 ($n = 9$), 60–89 ($n = 11$) and 90–123 ($n = 10$) years old. The annual precipitation in the area ranges from 450 to 550 mm (Solantie 1987) and the temperature sum (above the threshold value of 5°C) from 1000 to 1200 d.d. (Helminen 1987).

The most abundant soil types (Anon. 1988) were Carbic Podzols ($n = 10$) and Dystric Gleysols ($n = 7$). The remaining sites were classified as Cambic Podzols ($n = 5$), Ferric Podzols ($n = 4$), Haplic Arenosols ($n = 3$), and Dystric Regosols ($n = 1$) (Merilä & Ohtonen 1997). The thickness of the organic layer varies from 2.1 to 13.5 cm. The majority (75%) of the sites were classified as stony or very stony till soils (Viro 1952). The dominant particle size was silt, fine sand and medium sand in 30%, 33% and 33% of the sites studied, respectively.

The crown condition of the spruces was investigated during the summer of 1992 by estimating defoliation and needle discoloration (Anon. 1989). Defoliation was assessed on the upper half of the living crown in 10% classes. Discoloration was estimated on a 4-point scale: 0–10%, 11–25%, 26–60% or 61–100%. Plot mean defoliation and discoloration percentages were calculated using the mid-point of each class. The level of defoliation in the study area was compared with that of corresponding forest site types and stand age classes in southern Finland (demarcation line along latitude 65°). Data for this comparison were obtained from the results of the annual monitoring of forest condition (Anon. 1993). The total number of trees studied in the coastal area was 580 (in stand age classes 38–59 years ($n = 206$), 60–89 ($n = 223$), and 90–123 ($n = 151$)). The corresponding numbers of trees in the southern Finland material were 911, 200, 407, and 304.

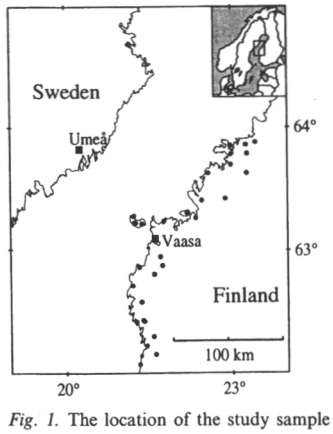

Fig. 1. The location of the study sample plots.

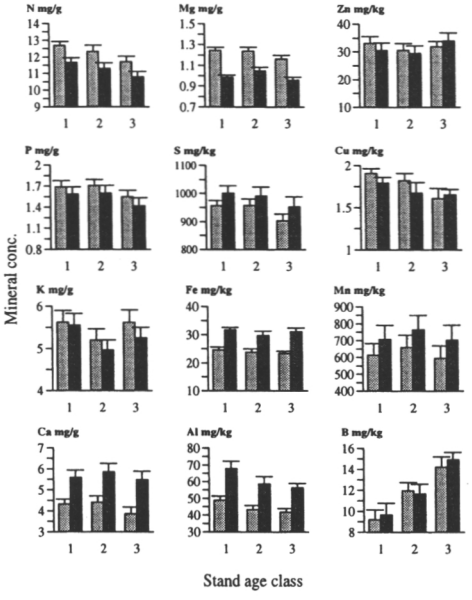

Fig. 2. The average elemental concentrations in the current needles (□) and previous year's needles (■) by stand age class: class 1 = 38–59 years old ($n = 9$), class 2 = 60–89 years old ($n = 11$), and class 3 = 90–123 years old ($n = 10$). Norway spruce stands were sampled in 1992. The error bar is the standard error (SE).

Tree-specific samples of current (C) and one-year-old (C + 1) needle age classes were collected from 10 trees on each sample plot in December 1992. Needle samples were collected from the top third of the southern side of the crown. Composite samples were formed for each sample plot and needle age class by mixing 1 g of dried powdered needle sample from each tree. The elemental concentrations of the needles were determined as described by Raitio (1991).

For the chemical analysis of the organic layer, 28 subsamples from the upper 5–7 cm of the organic layer were taken systematically with a stainless steel soil auger (diameter 58 mm) on 5–8 June 1994. In the laboratory, the samples were mixed by hand over an ice bath, and litter and roots >1 mm in diameter removed.

Moisture of the organic layer samples was determined. Total (tot) C and N were determined using a

Table 1. *Mean, standard error of the mean (SE), minimum and maximum values for stand and organic layer characteristics of the studied Norway spruce stands (n = 30)*

	Mean	SE	Min	Max
Stand characteristics				
Stand age, years	78	4.1	38	123
Crown defoliation, stand mean (%)	23.4	2.3	5.3	45.8
Needle discoloration, stand mean (%)	3.6	0.56	0.0	15.3
Organic layer characteristics				
Loss of ignition (OM), %	79.0	2.04	49.4	94.6
Field moisture, g g^{-1}OM	2.04	0.05	1.54	2.82
pH	4.03	0.04	3.63	4.48
N, mg g^{-1}OM	16.7	0.44	12.8	23.7
NH_4-Acet. (pH 4.65) extractable, mg kg^{-1}OM				
P	180	19	46	370
S	150	5	110	220
K	730	34	310	1190
Mg	520	29	350	1170
Ca	2600	183	630	4670
Mn	200	40	28	920
Fe	120	36	3	780
Al	160	38	8	940
Zn	30	4	13	120
Cu	0.58	0.01	0.46	0.85

CHN 600
LECO ~~TGA 500~~ analyzer and organic matter (OM) as ignition loss (485°C, 4 h). pH was measured after a subsample had been suspended in deionized water overnight (sample/water suspension = 1 : 3 v/v). Phosphorus, S, K, Mg, Ca, Mn, Fe, Al, Zn and Cu were extracted (ext) with NH_4OAc buffered to pH 4.65 using a soil:solution volume ratio of 1 : 10 (Halonen et al. 1983) and analyzed by an inductively coupled plasma emission spectrophotometer (ICP, ARL 3580). All concentration data have been converted to a dry organic matter basis.

Principal component analysis (PCA), based on the correlation matrix (Jongman et al. 1987), was used to create an index of site fertility. The input variables in PCA were: N in current needles (N_C), B in one-year-old needles (B_{C+1}), organic layer total N (N_{tot}), extractable S (S_{ext}), pH, and moisture content at the time of sampling. The site scores along the first PCA axis were used as the site fertility index.

RESULTS

The elemental concentrations in C and C + 1 needles by stand age class are presented in Fig. 2. Applying the limit value of 12.5 g kg^{-1} to N concentrations of C needles (Jukka 1988), 53% of the stands suffer from N deficiency. Current and previous year's needle Cu concentrations in all stands were according to Ahrens (1964), low (Fig. 2). They were highly correlated to N concentrations ($r_S = 0.82$ and $r_S = 0.81$ in C and C + 1 needles, respectively).

Crown defoliation and discoloration were clearly associated with stand age (Tables 1, 2). Frequency distributions of tree-specific defoliation in the study area differed significantly from that in southern Finland in the 60–89 and 90–125 years old stand age classes ($\chi^2 = 24.89$, $p < 0.001$ and $\chi^2 = 41.33$, $p < 0.001$, respectively). The spruces in the study area showed a higher level of defoliation and this occurred in younger stands than elsewhere in southern Finland (Fig. 3).

All significant correlations between crown defoliation, discoloration, stand age, needle elements and organic layer properties are presented in Table 2. Stand age, defoliation and discoloration were negatively correlated with needle concentrations of N and Cu, and positively correlated with that of B. In addition, stand age was in weak negative correlation with foliar S and Al concentrations. Of the organic layer chemical properties (Table 1), soil N_{tot} and S_{ext} showed a negative correlation with stand age and with crown defoliation and discoloration.

The first PCA axis indicates increasing fertility and decreasing soil moisture gradient. On this axis, needle

Table 2. *Significant Spearman rank correlation coefficients between crown defoliation, discoloration, stand age, needle elements and soil properties in spruce stands studied (n = 30)*

	Crown defoliation	Crown discoloration	Stand age
Crown discoloration	0.786***		
Stand age	0.809***	0.605***	
Needle			
N_C	−0.420*	−0.550**	−0.385*
N_{C+1}	−0.397*	−0.571***	−0.354*
S_C	NS	NS	−0.306*
B_C	0.548**	0.451**	0.649***
B_{C+1}	0.621***	0.368*	0.696***
Cu_C	−0.463**	−0.515**	−0.473**
Cu_{C+1}	−0.362*	−0.452**	−0.310*
Al_C	NS	NS	−0.413*
Al_{C+1}	NS	NS	−0.321*
Organic layer			
N_{tot}	−0.411*	−0.487**	−0.507**
S_{ext}	−0.505**	−0.471**	−0.422*
Ca_{ext}	NS	−0.377*	NS

Levels of significance: * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), NS (no significance).

N_C, soil N_{tot}, S_{ext}, and pH had positive loadings, whereas needle B_{C+1} and field moisture had negative loadings (Table 3). Site scores along axis 1 (= "site fertility index") correlated negatively with stand age ($r_S = -0.66$, $p < 0.001$; Fig. 4a), defoliation ($r_S = -0.67$, $p < 0.001$; Fig. 4b) and discoloration ($r_S = -0.68$, $p < 0.001$; Fig. 4c).

Stand-age-adjusted partial correlations of needle elements and of soil properties with crown defoliation and discoloration were insignificant, with the exception of that between site fertility index and crown discoloration ($r = -0.41$, $p = 0.03$, $df = 27$).

Crown condition of old stands growing on Dystric Gleysols or Carbic Podzols was the worst (Figs. 4a–c). Hence, there was a tendency toward crown condition related needle elements being either lower (N, Cu) or higher (B) on these two soil types, but these differences were usually indicative ($0.05 < p < 0.10$). In addition, the organic layers had higher moisture contents (g g^{-1}OM; Kruskall-Wallis $H = 14.5$, $p = 0.002$, $df = 3$) and the stands were more discolored ($H = 9.3$, $p = 0.03$, $df = 3$) on Dystric Gleysols than on Cambic Podzols. Furthermore, Carbic Podzols and Dystric Gleysols differed from Cambic Podzols and from Ferric Podzols with lower pH ($H = 11.1$, $p = 0.01$, $df = 3$).

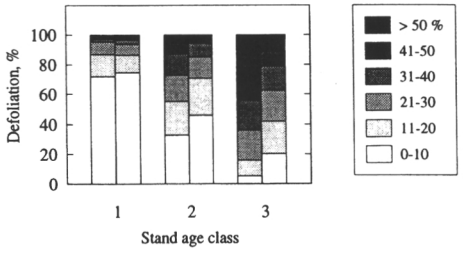

Fig. 3. The frequency distributions of trees among defoliation classes by stand age class (cf. Fig. 2) in the studied coastal Norway spruce stands (left) and southern Finland (right).

DISCUSSION

The worst crown condition was found in old stands growing on periodically waterlogged soil types (Dystric Gleysols and Carbic Podzols). This result might indicate that either: changes in microclimate and in nutrient cycling during succession of spruce forest lead to paludification (Sirén 1955) and to a deterioration in site fertility (Ranger & Nys 1994), or stands growing on infertile, paludified sites are not conducive to felling and regeneration activities and are therefore left uncut, or both. The fact that the spruce stands older than 60 years were more defoliated in the studied coastal area than elsewhere in southern Finland indicates that the crown condition of spruce in coastal areas is affected by other factors in addi-

Table 3. *Loadings of the axis 1 of the Principal Component Analysis*

	PCA Axis 1 increasing fertility, decreasing moisture
Needle	
N_C	0.50
B_{C+1}	−0.26
Organic layer	
N_{tot}	0.50
S_{ext}	0.46
pH	0.28
Moisture content	−0.37
Variance explained (%)	37.0

tion to stand age. Moreover, the principal component scores along axis 1, which we considered to reflect site fertility, were significantly correlated with crown discoloration even after adjustment for stand age.

Waterlogged, anaerobic soil conditions impede the uptake of water and mineral nutrients (Marschner 1995), especially NH_4-N (Lévy 1981), which is the dominant form of mineral N in acid and occasionally waterlogged soil conditions, where net NO_3-N accumulation is low (Smolander et al. 1995, Stark & Hart 1997). The nitrogen deficiency of spruce stands studied might thus be due to impaired N uptake. In addition, the slow decomposition of organic matter caused by periodic excess soil moisture, acid soil, lignin-rich needle litter and soil climate evidently results in the immobilization of essential nutrients, especially N, in forest floor (Flanagan & Van Cleve 1983, Merilä & Ohtonen 1997).

One factor that probably affects forest condition in the study area is low soil temperature or even soil frost. Frost depth is inversely related to the thickness of the snow cover (Soveri & Varjo 1977). The study area typically has only a thin snow cover (Solantie 1987) and hence deep, long-lasting soil frosts develop (Huttunen & Soveri 1993). Because of efficient canopy interception in a closed spruce stand, the snow cover is thinner than elsewhere and the soil frost persists for longer (Yli-Vakkuri 1960, Mustonen 1966). Spruce stands in this area are thus susceptible to desiccation injuries during sunny spring days when transpiration increases as the leaf temperature rises above freezing, but water cannot be absorbed through the roots at a fast enough rate to replace transpirational losses (Kozlowski et al. 1991). Low soil temperatures in the beginning of the growing season may also impair N uptake (Karlsson & Nordell 1996) and root growth (Mackay & Barber 1984).

Of all the measured mineral elements in needles, B had the strongest relation to crown condition, being positively correlated with crown defoliation. Raised foliar B concentrations have been observed earlier in studies concerning the crown reduction of Scots pine caused by browsing moose (Löyttyniemi 1985), Scleroderris canker (*Gremmeniella abietina* (Lagerb.) Morelet) infections or manual pruning (Nuorteva & Kurkela 1993). In the contrast, Frey & Frey (1995) reported a slight decrease in B concentrations in defoliated spruce trees. The transpiration stream primarily governs distribution of B in the plants: the regulation of B uptake and translocation is rather limited in comparison to that of other mineral nutrients (Marschner 1995). It thus might be assumed that raised B concentration in trees with reduced needle mass is due to its accumulation in remaining needles of a defoliated crown as a consequence of the xylem water flow.

The needle concentrations of Cu were negatively correlated with crown defoliation and discoloration. Needle Cu concentrations were also clearly positively correlated to needle N concentrations. Our results suggest that, in the spruce stands studied, the relation between N availability and Cu uptake may be placed in the ascending part of the optimum curve, presented for some tree species by Van den Burg (1983). The low Cu concentration in needles might thus partly be due to poor N supply of the stands. On the other hand, adsorption of Cu to organically complexed forms may affect Cu availability (Stevenson & Fitch 1981) as well as low soil temperature, which has been shown to affect strongly to desorption of Cu (McLaren et al. 1990). Low needle Cu in spruce stands has been observed also elsewhere in Finland, both on peatlands (Silfverberg 1980) and on mineral soils (Raitio 1994). However, the visible Cu deficiency symptoms described by Van Goor & Henkens (1966) and Van Goor (1968) have not been observed. This suggest that Norway spruce in Finland might be unsusceptible to Cu deficiency symptoms. Similar findings have been made in Netherlands in studies concerning Norway spruce growing on acid humus podzols with low Cu availability (Van den Burg 1982). Thus, the reported Cu deficiency limit of 3.5 mg kg^{-1} dwt. (Ahrens 1964) appears to be too high, at least for sites with a low N supply.

The results suggest poor crown condition of the coastal spruce stands to be related with natural soil

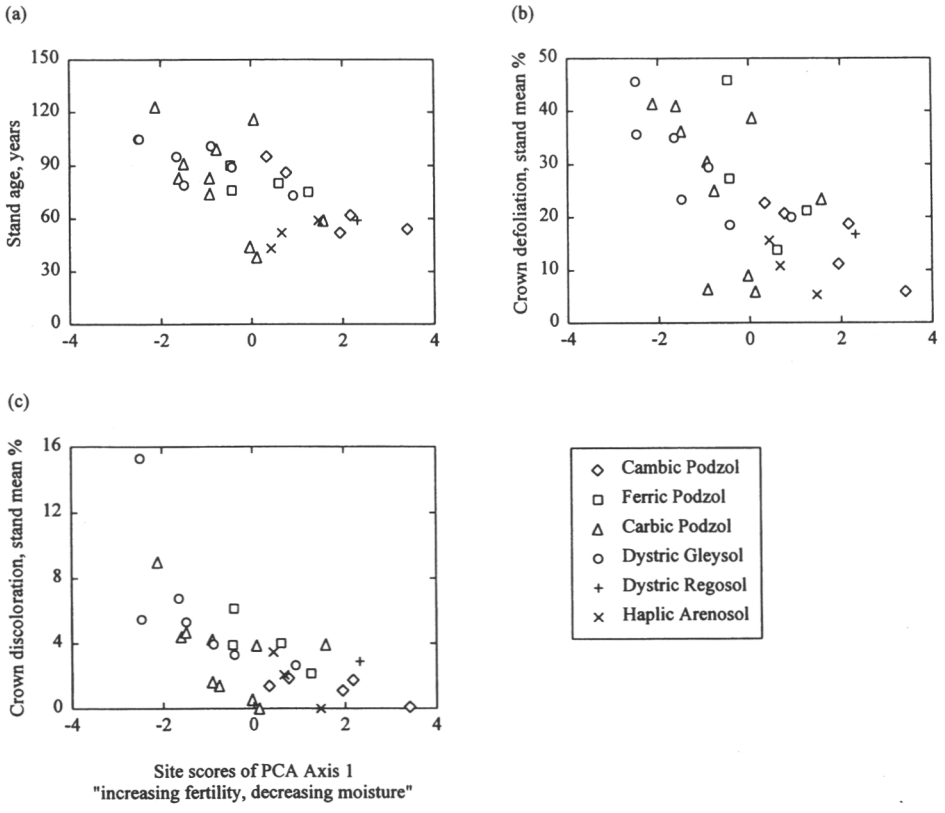

Fig. 4. Relationship between site fertility index (increasing fertility, decreasing moisture; site scores of PCA axis 1) and (a) stand age, (b) crown defoliation, and (c) crown discoloration in the coastal Norway spruce stands ($n = 30$) studied.

factors such as periodic water-logging and low fertility. Long-lasting soil frost as a consequence of thin snow cover might be an additional stress factor impeding water and nutrient uptake in the beginning of the growing season.

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Paper II

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II

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ORIGINAL PAPER

P. Merilä · R. Ohtonen

Soil microbial activity in the coastal Norway spruce [*Picea abies* (L.) Karst.] forests of the Gulf of Bothnia in relation to humus-layer quality, moisture and soil types

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Abstract Relationships between chemical, physical and microbial properties in the humus layer, soil type and crown condition of Norway spruce (*Picea abies*) were studied in stands located along the Finnish and Swedish coasts of the Straits of the Gulf of Bothnia. Humus layers of ferric podzols had higher basal respiration (BASAL) than those of carbic podzols and higher substrate-induced respiration (SIR) than those of both carbic podzols and dystric gleysols. BASAL and SIR correlated with environmental factors associated with humus-layer fertility; i.e. they were positively associated with pH and extractable K, Mn and P and negatively associated with organic-matter content of the humus layer. A short lag-time was associated with humus-layer fertility and a long lag-time with crown defoliation. Specific respiration increment was lowest at sites with high field moisture and discoloured crowns. The results suggest that on carbic podzols and dystric gleysols poor nutrient status, acidity and lack of oxygen due to excess moisture in the humus layer result in low microbial activity and poor condition of spruce.

Key words Microbial activity · Forest soil · Nutrients · Land uplift · *Picea abies* · Substrate-induced respiration

Introduction

For several decades, attention has been paid to the defoliated and discoloured Norway spruce [*Picea abies* (L.) Karst.] forests located along the Straits of the Gulf of Bothnia, especially on the Finnish side (Appelroth 1948). This area is characterised by uplifting of land (8–9 mm a^{-1}; Mäkinen et al. 1986), which continuously provides new surface for invasion of plant communities. During succession, Norway spruce becomes the dominant tree species following the alder-dominated (*Alnus incana*) deciduous vegetation of the shoreline (Appelroth 1948). Spruce stands of this region are generally nitrogen-poor and probably also phosphorus-poor, with relatively thick humus layers (Raitio 1996), indicating immobilisation of nutrients in the forest floor. Microbial activity in soil is thus of special importance, as it plays an essential role in the nutrient cycling and reflects the moisture, aeration (Blume et al. 1991), temperature (Mikola 1960; Van Cleve et al. 1990), nutrient status (Berg and Tamm 1991) and composition of organic matter in the humus layer (Wardle 1992).

In this study we examined microbial activity in the humus layer of Norway spruce stands with various soil types and with different soil nutrient and moisture status. Microbial activity in relation to crown condition was also evaluated.

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Materials and methods

Description of sites and crown condition

The Norway spruce stands (n = 42) located along the Straits of the Gulf of Bothnia in Finland and Sweden (Raitio 1996; Fig. 1) are classified as *Myrtillus* (mesic) or *Oxalis-Myrtillus* (herb-rich) forest site types according to the Finnish site-type classification (Cajander 1949). The condition of the spruce crowns was estimated in terms of defoliation and needle discolouration. The former was recorded as the site-specific mean percentage of defoliation in the dominant crown layer and the latter as the proportion of spruce trees with more than 5% of the needles discoloured (UN/ECE 1994; Raitio 1996; Table 1).

Soil description, sampling and analyses

The soils were described by determining the thickness of the humus layer, the soil type (FAO 1988) and the humus type, classified as mor, moder, undisturbed peat layer or disturbed peat layer.

For chemical and microbiological analyses, 28 subsamples from the upper 5–7 cm of the humus layer were taken systematically with

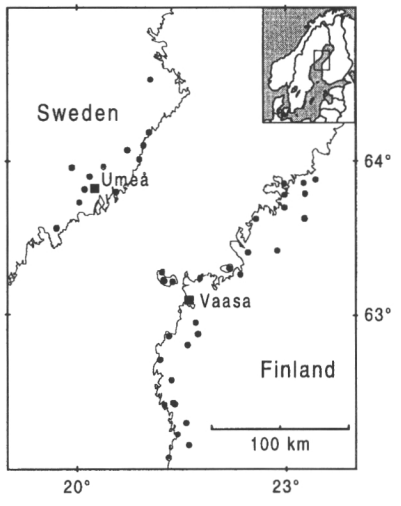

Fig. 1 Location of the sample plots

Table 1 Mean, standard error of the mean (SE), minimum and maximum values for stand and humus layer characteristics of the studied Norway spruce stands (n = 42) entered in redundancy analysis (*OM* organic matter content)

	Mean	SE	Min	Max
Stand characteristics				
Stand age, years	84	3.7	38	135
Crown defoliation, stand mean %	19.3	1.5	5.0	41.7
Percentage of spruce with >5% of the needles discolored	16.6	3.0	0.0	70.0
Humus layer characteristics				
Thickness of the humus layer, mm	76	5.3	21	170
Loss of ignition (OM), %	80.2	1.77	48.3	94.4
Initial water content (field moisture), g g^{-1}OM	2.06	0.04	1.55	2.82
pH	4.01	0.03	3.63	4.48
N, mg g^{-1}OM	16.0	0.4	12.2	23.7
NH_4-Ac (pH 4.65) extractable, mg kg^{-1}OM				
P	180	15	46	370
S	150	5	99	220
K	760	29	310	1190
Mg	480	23	290	1170
Ca	2500	141	630	4670
Mn	180	30	28	920

a stainless steel soil auger (diameter 58 mm) on 5–8 June 1994. The subsamples were pooled, sieved and kept frozen until analysed.

Samples were dried at 50°C for chemical analyses. Total C and N were determined with a LECO TGA-500 analyser. pH was measured after the sample had been suspended overnight (sample : water suspension = 1:3 v/v). P, S, Ca, Mg, K, Al, Fe and Mn were extracted with NH_4OAc buffered to pH 4.65 using a soil:solution volume ratio of 1:10 (Halonen et al. 1983) and analysed by an inductively coupled plasma emission spectrophotometer (ICP, ARL 3580). All concentration data were converted to an organic-matter basis.

Moisture (95°C overnight) and organic-matter content (OM; 485°C, 4 h) of soil samples were determined. The samples were moistened to a water content that equalled 250% of the OM, and 4–6 replicates were analysed for microbial variables using a respirometer (Nordgren 1988). After the beginning of the experimental run, basal respiration (BASAL) was analysed for at least 40 h at a stable rate of respiration, after which 200 mg glucose, 22 mg N as $(NH_4)_2SO_4$ and 2.4 mg of P as KH_2PO_4 were added with vigorous shaking of the sample. Substrate-induced respiration (SIR) as the average CO_2 evolution over 3–6 h after addition of substrate, lag-time (Lag), and specific respiration increment (μCO_2) in the microbial community were then ascertained. μCO_2 is assumed to be comparable to the growth rate of the microbial community (Nordgren et al. 1988). The metabolic quotient of the soil microbes (qCO_2; Anderson and Domsch 1985a, b) was calculated as the relationship between BASAL and microbial biomass, which in turn was calculated from SIR values using the equation of Anderson and Domsch (1978).

Statistical analyses

Differences between soil types were studied by analysis of variance and paired means were tested using Tukey's test. In the case of unequal variances between groups, a Kruskall-Wallis nonparametric test was used and the mean ranks were compared pairwise. Because there were so few replicates, cambic podzols and haplic arenosols were omitted from the comparison.

The relationships between variables for microbial activity and site and humus-layer characteristics were investigated by redundancy analysis (RDA), which is a multivariate linear method and a canonical form of principal components analysis (Jongman et al. 1987). RDA analyses were performed on a correlation matrix with correlation biplot scaling of ordination scores using CANOCO version 3.10 (Braak 1990). Variables describing soil microbial activity, i.e. BASAL, SIR, Lag, μCO_2 and qCO_2, were entered as dependent variables ("species") in the program; and humus-layer chemistry and site variables (Table 1) were entered as independent (environmental) variables. Crown defoliation, thickness of the humus layer, field moisture, total N, and extractable Mg and Mn were transformed logarithmically; and OM and needle discolouration were arcsin-transformed in order to normalise their distributions.

Results

Soil and humus layer

The Finnish side of the region was dominated by carbic podzols and dystric gleysols, whereas on the Swedish side ferric podzols were more abundant (Table 2). The predominate type of humus layer was mor (64% of the stands), moder and undisturbed or disturbed peat layers being less common (7, 17 and 12%, respectively). The humus layers of the ferric podzols had higher pH ($F = 4.17$, $P = 0.025$, $df = 2$) and lower OM (Kruskall Wallis $H = 10.6$, $P = 0.005$) than those of the carbic podzols (Table 3). Furthermore, the humus layers of the ferric podzols had higher K concentrations ($F = 4.44$, $P = 0.02$, $df = 2$) than those of the dystric gleysols, which had the highest field moisture ($F = 6.34$, $P = 0.005$, $df = 2$) (Table 3).

Table 2 Soil-type distribution of the sample plots

	Cambic podzol	Ferric podzol	Carbic podzol	Dystric gleysol	Dystric regosol	Haplic arenosol	Total
Finland	5	4	10	7	1	3	30
Sweden	0	8	4	0	0	0	12
All	5	12	14	7	1	3	42

Table 3 Mean and standard error of the mean (SE) of some humus layer characteristics on the dominant soil types. According to Tukey's test (rejection level 0.05) the groups labelled with the *same letter* do not differ significantly. In the case of Kruskall-Wallis nonparametric test, significant difference is indicated by *small letters* (*OM* organic matter content)

	Ferric podzol (n = 12)			Carbic podzol (n = 14)			Dystric gleysol (n = 7)		
	Mean	SE		Mean	SE		Mean	SE	
Loss of ignition (OM), %	74.6	3.8	a	88.3	1.2	b	78.6	4.2	ab
pH	4.12	0.07	A	3.91	0.04	B	3.98	0.07	AB
Initial water content, g g^{-1}OM (field moisture)	2.07	0.05	A	2.02	0.05	A	2.36	0.12	B
Extractable K, mg kg^{-1} OM	850	53	A	710	33	AB	640	69	B

Table 4 Mean and standard error of the mean (SE) of microbial variables on the dominant soil types. According to Tukey's test (rejection level 0.05), the groups labelled with *same letter* do not differ significantly (*OM* organic matter content, *mic* microbes)

	Ferric podzol (n = 12)			Carbic podzol (n = 14)			Dystric gleysol (n = 7)			Total (n = 42)	
	Mean	SE		Mean	SE		Mean	SE		Mean	SE
Basal respiration, mg CO_2 g^{-1} OM h^{-1}	0.047	0.004	A	0.035	0.002	B	0.038	0.003	AB	0.040	0.002
Substrate-induced respiration, mg CO_2 g^{-1} OM h^{-1}	0.101	0.006	A	0.078	0.003	B	0.076	0.005	B	0.085	0.003
Lag time, h	18.7	1.2	A	20.1	1.1	A	20.0	1.0	A	19.8	0.5
Specific respiration increment	0.043	0.0012	A	0.041	0.0006	A	0.039	0.0019	A	0.0415	0.0006
Metabolic quotient, mg C $mg^{-1}C_{mic}$ h^{-1}	0.0066	0.0002	A	0.0067	0.0004	A	0.0070	0.0005	A	0.0067	0.0002

Microbial activity

The humus layers of the ferric podzols had higher BASAL (F = 4.17, P = 0.025, df = 2) and SIR (F = 7.88; P = 0.002, df = 2) than the carbic podzols and higher SIR than the gleysols (Table 4). The differences between humus types in the variables for soil microbial activity were not large enough to be statistically significant.

The organic soils studied here were quite heterogeneous. RDA analysis was thus performed not only for the whole material (n = 42; Fig. 2), but also separately for mor samples (n = 27), for samples from the Finnish side (n = 30) and for samples that had no extreme values for the environmental variables (n = 40). In all cases, the basic structure of the RDA solution remained nearly the same. The first two axes of the RDA solution for the whole material (Fig. 2) explained 56% of the variation in the microbiological variables, the eigenvalues being 0.386 and 0.171 for the first and second axes, respectively. All inflation factors were less than 7. BASAL, SIR and Lag were best fitted on axis 1, with the amount of variance explained for each of them being 73.4, 61.8 and 53.3%, respectively. μCO_2 was best fitted on axis 2, which explained 68.8% of its variance. qCO_2 also had the best fit on axis 2, but it explained only 12.3% of the variance of qCO_2.

BASAL and SIR were correlated with environmental factors associated with humus layer fertility; i.e. positively with pH and extractable K, Mn and P, but negatively with OM (Fig. 2). Lag was negatively correlated with fertility of the humus layer. μCO_2 showed a pattern that was somewhat different from that of the other microbial activities, being negatively correlated with field moisture and OM, but positively correlated with pH and extractable Ca, Mn and P. qCO_2 was situated near the centre of the ordination space, thus indicating poor correlation with the environmental variables.

Total N, extractable Mg and S, and thickness of the humus layer were situated near the centre of the ordination space, thus showing a poor correlation, if any, with variables for microbial activity. No correlations were found between microbial activity and stand age. Lag and crown defoliation were positively correlated, while μCO_2 and needle discolouration were negatively correlated with each other.

Discussion

Both carbic podzols and dystric gleysols are soil types that develop on moist sites. Thus, excess water, which results in anaerobic conditions, may be the reason for smaller microbial biomass on these soil types (Blume et al. 1991); this interpretation is supported by the fact that in the present study the field moisture was highest in gleysols. The humus layers of carbic podzols had lower pH than those of the ferric podzols, which is mostly the result of higher levels of OM and thus of high organic-acid content. On the other hand, under wet, anaerobic conditions, which occasionally prevail in the humus layers of carbic podzols, the fermentative microbes themselves increase soil acidity through the production of organic acids (Killham 1994). The high OM content of the humus layers of carbic pod-

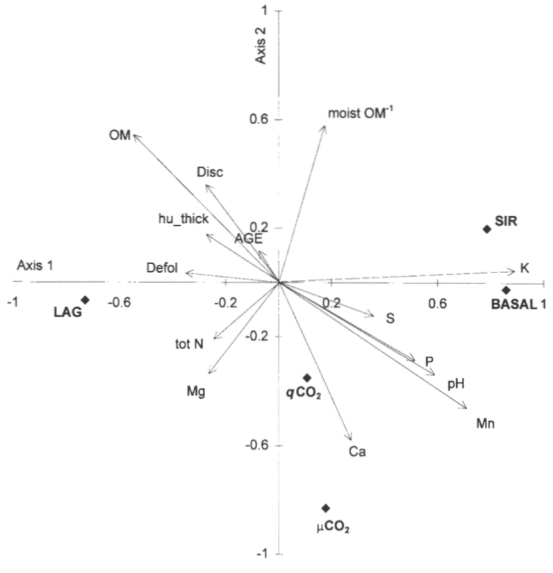

Fig. 2 Scores for microbial activity (◆) and environmental variables (→) along redundancy analysis ordination axes 1 and 2. All concentration data were converted to an organic-matter basis (*BASAL* basal respiration, *SIR* substrate-induced respiration, *LAG* lag time, μCO_2 specific respiration increment, qCO_2 metabolic quotient, *Def* mean percentage of crown defoliation in the dominant crown layer, *Disc* proportion of spruces with more than 5% of the needles discolored, *huthick* thickness of the humus layer, *AGE* stand age, *OM* organic matter content of the humus layer, *moist* OM^{-1} initial water content per organic matter content; *K*, *Ca*, *Mg*, *Mn*, *P*, *S* concentrations of ammonium acetate (pH 4.65) extractable elements in the humus layer, *tot N* total nitrogen concentration in the humus layer)

zols also indicates unfavourable conditions for soil fauna, which are important in mobilising nutrients bound in the forest floor (Setälä et al. 1990).

The higher the content of organic matter in the humus layer, the higher is the soil moisture and the poorer are the fertility and microbial activity. Low concentrations of nutrients can be considered to reflect a low rate of mineralisation; but on the other hand, decomposition may also be limited by acidity and lack of nutrients (e.g. Bockheim et al. 1991). This interdependence complicates interpretation of the correlations between microbial activity and nutrient concentrations in the soil. The essential nutrients N, P and S are susceptible to binding in the organic layer, and the total amounts are not clearly related to microbial activity, as was shown in the case of total N in our study.

Extractable P was positively correlated with BASAL and SIR, was also slightly correlated with μCO_2 and was negatively correlated with Lag. P has been thought to be a limiting nutrient for decomposers, although not as limiting as N in coniferous forests (e.g. Staaf and Berg 1982; Nordgren 1992). A pattern very similar to that of the microbial-activity variables (BASAL, SIR, Lag, μCO_2) and P was observed between those variables and soil pH, the pH range in this material being 3.63–4.48.

BASAL and SIR correlated positively and Lag correlated negatively with K. K is highly water soluble and, of all the elements studied, has been found to be lost most rapidly from the needle litter (Staaf and Berg 1982; Bockheim et al.1991), thus showing that K did not limit microbial growth. Correlation with microbial activity and K may reflect the proportion of mineral soil particles in the humus layer, which increases with fertility. Microbial activity was also quite highly correlated with Mn. The main transfer pathway of both these elements is throughfall (Helmisaari 1995). K and Mn might be correlated with easily decomposable organic substances leached from the canopy, which may contribute to microbial activity. Mn has been shown to be essential for the activity of the lignolytic enzymes (Archibald and Roy 1992; Perez and Jeffries 1992), and microbial activity may even be regulated by the availability of Mn (Berg et al. 1995).

Our results suggest that poor nutrient status, acidity and lack of oxygen due to excess moisture in the humus layer all result in low microbial activity, especially on carbic podzols and dystric gleysols. Low rate of mineralisation may contribute to the nutrient deficiency and poor crown condition of the spruce forests in the Gulf of Bothnia region in a similar manner to spruce forests on raw humus sites in northern Finland where cold, humid climate and recalcitrant needle litter result in low microbial activity and immobilisation of nutrients in the forest floor (Sirén 1955).

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Paper III

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III

PERGAMON

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Soil nitrogen transformations along a primary succession transect on the land-uplift coast in western Finland

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Abstract

We monitored net and gross N transformations in the organic layer along a primary successional transect (alder/rowan, birch, birch/spruce, spruce I and spruce II) typical for the land-uplift coast in Western Finland. The relationships between N transformations, vegetation succession and organic matter quality (i.e. concentration of dissolved forms of N, C/N ratio, moisture and acidity) were then evaluated. Net N mineralisation rates in the organic layer were estimated in 5-week incubation experiments in situ using intact soil cores, and in the laboratory on sieved, fresh organic layer samples. Microbial biomass N (fumigation–extraction) and gross N mineralisation (^{15}N-isotope dilution method), were determined once in the laboratory. The C/N ratio increased and pH and net N mineralisation decreased in the organic layer along the succession transect. The alder/rowan site was the only site to show net nitrification. Microbial biomass N tended to increase along the transect from the alder/rowan site to spruce I, and decreased again in spruce II. Concurrently, gross N mineralisation showed a tentative increasing trend along the transect, although the differences between the sites were non-significant. The higher net N mineralisation rates in the alder/rowan site compared to the spruce sites were thus due to lower microbial immobilisation rather than to greater gross N mineralisation. Possible methodological reasons for the lack of response of gross N mineralisation rate to decreasing soil organic matter quality are discussed.

Keywords: *Alnus incana*; *Betula* sp; Boreal; Forest soil; Nitrification; Nitrogen mineralisation; *Picea abies*

1. Introduction

Productivity of forest ecosystems in the boreal region is primarily governed by the supply of soil nitrogen, which is lacking in most parent materials. Therefore, in early stages of primary succession, dinitrogen-fixing colonizers play an extremely important role in accumulating a pool of soil nitrogen that facilitates the establishment of later successional species (Van Cleve et al., 1971; Chapin et al., 1994). As is generally the case in mature ecosystems (Odum, 1969), the nitrogen cycle in late successional stages of undisturbed boreal forest is relatively tight. The supply of nitrogen at this stage is largely controlled by the rate at which plant available N is produced from above- and below-ground litter via decomposition, ammonification and nitrification (Tamm, 1991). In addition to the rate of ammonification and nitrification, N availability to plants is influenced by the rate at which inorganic N is consumed in microbial immobilisation. In addition to the usage of inorganic N, mycorrhizal and non-mycorrhizal uptake of organic N have also been demonstrated (Kielland, 1994; Raab et al., 1996; Näsholm et al., 1998). However, the true significance of organic N sources in plant N nutrition has not been quantified in the field.

Due to isostatic rebound, the coastline along the Gulf of Bothnia between Finland and Sweden is continuously rising at a rate of 8–9 mm y^{-1} (Mäkinen et al., 1986), and new land is becoming exposed to the combined effect of soil formation (Starr, 1991) and other ecosystem processes that are controlled by the prevailing climate. Successional stages of forest ecosystems thus appear as a spatial continuum running at right angles to the coastline. The succession of forest vegetation on stony, fine-textured till soils starts from alder-dominated (*Alnus incana* (L.) Moench) deciduous shoreline vegetation, and ends in almost pure Norway spruce stands (Ericson, 1980), often via a birch-dominated (mostly *Betula pubescens* Ehrh.) intermediate stage. The ecological change from the dinitrogen-fixing alder stage to often paludified and nitrogen deficient spruce stands with a

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thick humus layer is considerable (Merilä et al., 1998). A chronosequence of this kind offers an opportunity to study the interrelationship between vegetational succession and microbial processes affecting N availability to plants. In the coastal region along the Gulf of Bothnia, such studies are scarce (Aikio et al., 2000), but comparable successional ecosystems have been studied intensively in Alaska at Glacier Bay National Park (Bormann and Sidle, 1990; Chapin et al., 1994) and in the Tanana river floodplain (Klingensmith and Van Cleve, 1993; Van Cleve et al., 1993; Clein and Schimel, 1995; Schimel et al., 1998). The occurrence of N_2-fixing *Alnus* species in the early stages of primary succession and, on the other hand, the dominance of *Picea* species in late successional stages, are probably the most distinct common features between primary successional seres in Alaska and the Gulf of Bothnia.

In chronosequence studies conducted in Alaskan conditions, the rate of net N mineralisation has been shown to decline with advancing succession from the poplar–alder (*Populus balsamifera–Alnus tenuifolia* (Nutt.)) stage towards mature white spruce (*Picea glauca* (Moench) Voss) stands (Klingensmith and Van Cleve, 1993; Van Cleve et al., 1993). Above-ground net primary productivity of *Picea* was shown to decrease by 50% over a 160-year *Picea* dominated portion of a chronosequence studied by Bormann and Sidle (1990). The changes in nitrogen availability and, hence productivity, have been concluded to be related to changes in organic matter quality, through the control of microbial activity (Van Cleve and Yarie, 1986; Bormann and Sidle, 1990). Net N mineralisation was clearly related to significant increases in the lignin:N and C/N ratios in the organic layer, suggesting that early and mid-successional deciduous vegetation types produce litter that is less recalcitrant to decomposition in comparison to litter of the late successional coniferous forest stages (Van Cleve et al., 1993, 1996). Soil temperature also declined with advancing succession, but its relationship with net N mineralisation was not as clear as that between net N mineralisation and organic matter chemistry (Van Cleve et al., 1993). Plants may also affect N cycling by producing secondary compounds that directly influence microbial activity, acting as substrates, inhibitors or inducers (Van Cleve et al., 1991; Schimel et al., 1996, 1998; Pellissier and Souto, 1999). In the study of Schimel et al. (1996), for instance, balsam poplar tannins were found to act as general microbial inhibitors, while low-molecular-weight phenolics functioned as substrate for microbial growth. In addition, monoterpenes have also been found to inhibit N mineralisation (White, 1986) and nitrification (White, 1986; Paavolainen et al., 1998) in coniferous forest soil.

In this study, we monitored changes in net and gross N transformations and microbial biomass N in the organic layer along a primary successional transect typical for the land-uplift coast in Western Finland in order to gain information about the processes that may control N availability. The relationships between N transformations, vegetation succession and organic matter quality (i.e. concentration of dissolved forms of N, C/N ratio, moisture and acidity) were then evaluated.

2. Materials and methods

2.1. Study area

The study was conducted along a primary successional transect (from NW to SE) in the archipelago of Raippaluoto in western Finland (63°20′N, 21°15′E). The transect is located in a nature reserve, and human impact on the development of the vegetation can be considered minor although some logging and sheep grazing might have occurred in the past. The transect comprises the following four forest sites (Fig. 1):

(1) *Alder/rowan*: 70-year-old alder/rowan stand (*Alnus incana* (L.) Moench and *Sorbus aucuparia* L.)
(2) *Birch*: 80-year-old birch stand (mainly *Betula pubescens* Ehrh.)
(3) *Birch/spruce*: 75-year-old birch/spruce stand (*B. pubescens* Ehrh., *B. pendula* Roth., and *Picea abies* (L.) Karst.)
(4) *Spruce I*: 95-year-old spruce stand (*P. abies*).

In order to extend the age sequence, a fifth forest site was chosen 12.2 km to the south of the transect:

(5) *Spruce II*: 130-year-old spruce stand (*P. abies*).

One sample plot (area 30 m × 30 m, except for the alder/rowan site 15 m × 40 m, see Fig. 1) was established in each of the forest stages. A number of stand and organic layer characteristics are presented for each plot in Table 1 and the coverage of selected plant species on each plot in Table 2. During the field incubation experiments, temperature was measured in the organic layer of the alder/rowan and spruce II sites with a Tinytalk®II datalogger at 5 cm depth (Fig. 2).

2.2. Sampling, experiments, and analyses

Net N mineralisation and nitrification rates in the organic layer were estimated in incubation experiments in situ using intact soil cores, and in the laboratory on sieved, fresh organic layer samples. In 1997, both experiments consisted of four consecutive 5-week incubation periods, called here Jun-97, Jul-97, Aug-97 and Sep-97. The corresponding incubation periods in the field were 3 June–8 July, 8 July–12 August, 12 August–16 September and 16 September–21 October, respectively. In 1998, the samples were collected once (13–14 July, called here Jul-98), and a 5-week incubation experiment in the laboratory similar to that in 1997 was repeated.

The method applied in the field incubation experiments was essentially the same as described by Tietema et al.

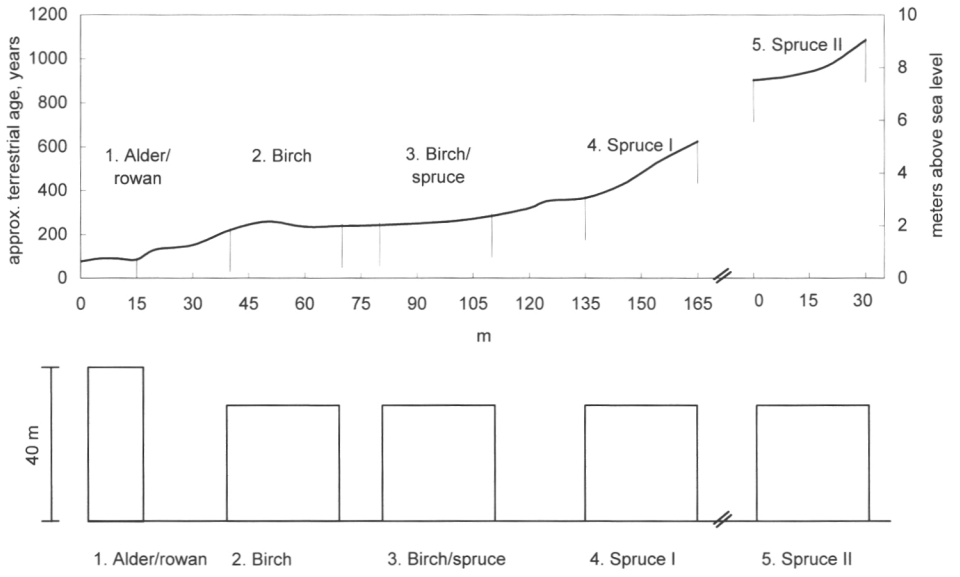
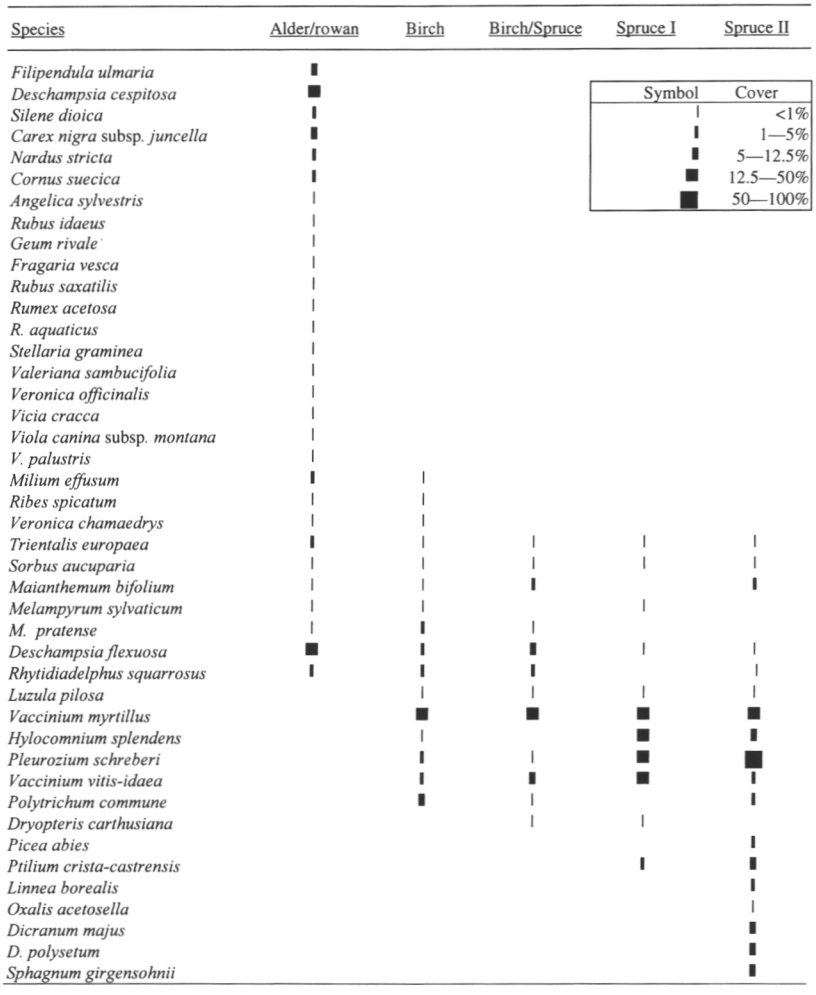


Fig. 1. The profile (above) and map (below) of the primary successional transect. *X* axis refers to both figures. Terrestrial age refers to the number of years elapsed since the site rose above sea level.

Table 1
Stand characteristics and thickness, loss in weight on ignition, pH($CaCl_2$), pH(H_2O), C/N ratio and total and extractable nutrients (Halonen et al., 1983) of the organic layer of the successional forest sites. Stand characteristics and C/N ratio ($n = 8$ for each stage) were measured in 1998. Values for thickness ($n = 96$), loss on ignition ($n = 24$) and pH ($n = 8$) of the organic layer are means of samples analysed in 1997. Total and extractable nutrients were analysed as duplicates from one composite sample/sampling in 1997 ($n = 4$). Different capital letters between the sites indicate significant differences according to Tukey's test (rejection level 0.05). Small letters are used for the comparison of mean ranks. SEMs are presented in parentheses

	Alder/rowan	Birch	Birch/spruce	Spruce I	Spruce II
Stand characteristic					
Stem number (ha^{-1})	1617	978	1411	589	1244
Mean diameter at breast height (cm)	13.0	23.4	20.4	26.4	22.1
Mean height (m)	7.5	14.1	14.7	16.8	16.0
Basal area ($m^2\ ha^{-1}$)	9.9	20.3	26.9	19.7	26.7
Stem volume ($m^3\ ha^{-1}$)	38.2	128.4	186.6	149.5	203.8
Organic layer					
Thickness (cm)	6.5	6.6	7.4	6.6	6.8
Loss in weight on ignition (OM) (%)	87.2 (1.2)	87.0 (1.5)	90.2 (0.7)	84.9 (2.0)	84.7 (1.9)
pH($CaCl_2$)	3.88 (0.10)a	3.23 (0.04)ab	3.10 (0.01)b	3.10 (0.03)b	3.04 (0.04)b
pH(H_2O)	5.11 (0.11)a	4.32 (0.06)ab	4.02 (0.03)bc	4.06 (0.04)bc	3.98 (0.03)c
C/N ratio	15.9 (0.5)A	20.2 (0.8)B	21.4 (0.6)B	31.7 (1.5)C	37.3 (1.3)D
Total nutrients					
N ($mg\ g^{-1}$ OM)	33.0	25.7	25.1	16.0	14.3
P ($mg\ kg^{-1}$ OM)	1310	1500	1690	1110	960
K ($mg\ kg^{-1}$ OM)	1050	940	860	950	810
Ca ($mg\ kg^{-1}$ OM)	4540	2800	1730	2980	3660
Mg ($mg\ kg^{-1}$ OM)	2740	1000	650	760	720
Cu ($mg\ kg^{-1}$ OM)	22.9	24.7	33.9	12.7	11.6
NH_4-Ac (pH 4.65) ext. nutrients ($mg\ kg^{-1}$ OM)					
P	200	240	160	350	200
S	150	180	200	160	140
K	840	850	750	880	650
Ca	3010	1900	1150	1980	2450
Mg	1920	760	480	550	480

Table 2
Cover of selected species in ground vegetation of the successional forest sites studied



(1990). Twenty-four soil cores, consisting of the whole organic layer and in a few cases also the attached uppermost part (max. 3 cm thick) of the mineral soil layer, were sampled systematically from each plot (sampling grid, see Fig. 1) with a stainless steel soil auger (diameter 7.3 cm) and placed in PVC tubes (diameter 6.7 cm). The tubes were sealed at the top and bottom with PVC lids and returned to the soil for incubation. Four holes in the upper part of the tubes ensured aeration. After 5 weeks, the samples were removed and any visible mineral soil, if present, was carefully removed. The organic layer samples from three adjacent cores were combined to give eight composite samples per plot. The samples were taken to the laboratory and kept cool (4 °C) until sieved and weighed. The moisture content (105 °C, 24 h), loss in weight on ignition (OM; 500 °C, 4 h) and pH (in 0.01 M $CaCl_2$ or water, 1:3 v/v, standing overnight) of the organic layer samples were determined.

At the start of each field incubation, 24 additional cores were taken in a similar fashion at a spot less than 0.5 m from each incubation spot, and three adjacent cores were again combined. These samples were immediately taken to the laboratory, sieved, weighed and analysed for moisture content, loss in weight on ignition and pH in the same way as for the field-incubated samples. For the laboratory incubation experiment, two subsamples (corresponding to 2 g of OM) were weighed and moistened to a water content equal to 250% of OM. One subsample, used as a reference in both the laboratory and field experiments, was immediately frozen. The other was incubated at 14 ± 1 °C for 35 d in a glass bottle (125 ml volume) sealed with a piece of aluminium

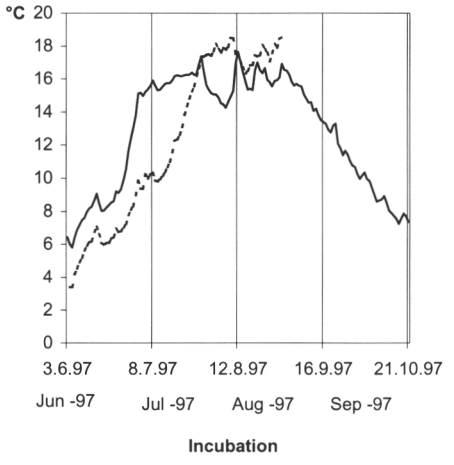


Fig. 2. Seasonal change in temperature at 5 cm depth in the organic layer beneath alder/rowan (solid line) and beneath spruce II (dash line) during field incubation experiments in 1997.

foil. The moisture content of the samples was adjusted with deionised water once a week during the incubation period. After incubation, the samples were kept frozen until analysed. In 1998, this procedure was repeated and one incubation experiment was carried out in the laboratory.

The reference samples and the samples incubated either in the field or in the laboratory were analysed for total dissolved nitrogen (TDN), NH_4–N and (NO_2 + NO_3)–N by extracting each sample (corresponding to 2 g OM) with 40 ml 1 M KCl for 2 h in a shaker. The extracts were diluted 1:1 and measured on a flow injection analyser (FIA Star 5020, Tecator). Net ammonification and nitrification in both the laboratory and field experiments were calculated by subtracting the initial NH_4–N and (NO_2 + NO_3)–N concentrations from the final (post-incubation) NH_4–N and (NO_2 + NO_3)–N concentrations, respectively. Net N mineralisation was calculated as the sum of net ammonification and net nitrification. The concentration of dissolved organic nitrogen (DON) was calculated by subtracting the NH_4–N and (NO_2 + NO_3)–N concentrations from the TDN concentration. The concentrations of TDN and DON for the Jun-97 incubation are missing.

Jul-98 samples were analysed for soil microbial biomass N (microbial N) using the fumigation–extraction method (Smolander et al., 1994), and for total C and N on a CN analyser (LECO CHN-600).

The net N mineralisation per unit area for the whole incubation period in the field was calculated on the basis of the net nitrification and ammonification per unit dry weight, the dry weight of the sieved samples, and the area of the soil cores in each incubation. No allowance was made for sieving losses and the presence of stones. Because total C and N were analysed for every subsample in Jul-98, these results were used in calculating the N mineralisation coefficient (Weier and MacRae, 1993) as the ratio: (net N mineralisation during the laboratory incubation)/(total N concentration in organic layer).

The gross N mineralisation in the laboratory was determined on sieved, fresh organic layer samples of Jul-98 using the isotope dilution method (Hart et al., 1994). Two subsamples (corresponding to 7.5 g OM) were weighed and 4 ml of a dilute $(^{15}NH_4)_2SO_4$ solution (0.04 g ammonium sulphate 98 atom% ^{15}N in 1 l distilled water) was injected (0.5 ml/injection) with a 19 gauge Microlance needle (Becton Dickinson and Co. Ltd, Drogheda, Ireland) into each sample. Before injection, the water content of the samples was equalised so that, after injection of ^{15}N, a water content of 250% of OM was reached. One subsample was immediately extracted with 1 M KCl to determine the recovery of $^{15}NH_4$. The other subsample was incubated for 3 days at +14.5 ± 0.5 °C in a glass bottle (125 ml volume) sealed with a piece of aluminium foil, after which it was extracted with 1 M KCl. The filtered extracts were kept frozen until analysed for TDN, NH_4–N and (NO_2 + NO_3)–N as described earlier. For the analyses of ^{15}N, 15 ml of fresh extractant was incubated for 3 days at +30 °C in airtight plastic jars together with a vial containing 0.01 M H_2SO_4, during which NH_4^+ was vaporised to NH_3 and captured in H_2SO_4 (Crooke and Simpson, 1971). The sulphuric acid was then dried at +45 °C, and diffusion and drying repeated four times on the same sample. The $^{15}N/^{15}N$ ratio was determined by mass spectrometry (Europa Scientific ANCA system) and the gross N mineralisation rates were calculated by the equation of Kirkham and Bartholomew (1954)).

In 1998, both fresh (gross N mineralisation experiment) and frozen (net N mineralisation experiment) organic layer samples were extracted for TDN, NH_4–N and (NO_2 + NO_3)–N. This made it possible to evaluate the effect of freezing on the concentrations of different forms of N.

2.3. Statistical analyses

Because this study was conducted on a single transect and successional stages were thus not replicated, statistical comparisons can be made to distinguish statistically significant differences between sites but not between the successional stages.

Differences in the variables between plots and between the 1997 incubations were tested with repeated measures analyses of a general linear model. In the case that the assumption of sphericity was not violated, contrasts were used to test the differences among variables between subsequent incubations (SPSS® version 9.0.1). The non-parametric Kruskal–Wallis test was used to distinguish differences between the sites, and pairwise differences were tested with Tukey's test. In the case that the equality of variances between the groups was not met, pairwise comparisons were made for mean ranks.

The Pearson (r) or the Spearman rank correlations (r_S)

Table 3
Pre-incubation field moisture (field and laboratory incubations), post-incubation field moisture (field incubation), initial concentrations of total dissolved nitrogen (TDN), NH_4–N, (NO_2 + NO_3)–N and dissolved organic nitrogen (DON) in incubation experiments in the organic layer of the successional forest sites. Mean and SEM (n = 7–8). Different capital letters between the sites indicate significant differences according to Tukey's test (rejection level 0.05). Small letters are used for the comparison of mean ranks of the groups. ND = not determined

	Incubation	Alder/rowan	Birch	Birch/spruce	Spruce I	Spruce II
Initial field moisture (g g^{-1} OM)	Jun-97	1.89 (0.16) B	2.78 (0.22) A	2.80 (0.20) A	1.85 (0.15) B	1.96 (0.20) B
	Jul-97	1.72 (0.08) B	2.33 (0.10) A	2.15 (0.09) A	2.22 (0.13) A	2.32 (0.11) A
	Aug-97	1.22 (0.08) D	1.90 (0.16) AB	1.75 (0.09) BC	1.46 (0.09) BC	2.20 (0.10) A
	Sep-97	2.31 (0.15) A	2.50 (0.10) A	2.49 (0.14) A	2.66 (0.21) A	2.73 (0.14) A
	Jul-98	1.75 (0.21) B	2.82 (0.21) A	2.57 (0.15) A	2.25 (0.20) AB	2.54 (0.18) A
Final field moisture (field incubation) (g g^{-1} OM)	Jun-97	1.83 (0.13) B	2.81 (0.17) A	3.02 (0.17) A	1.98 (0.20) B	1.86 (0.20) B
	Jul-97	1.75 (0.17) B	2.40 (0.09) A	2.29 (0.12) AB	2.53 (0.20) A	2.43 (0.09) A
	Aug-97	1.71 (0.14) B	2.42 (0.17) A	2.23 (0.10) AB	2.50 (0.20) A	2.64 (0.15) A
	Sep-97	2.10 (0.07) B	2.93 (0.17) A	2.85 (0.10) A	2.86 (0.16) A	2.87 (0.28) A
Initial TDN (mg kg^{-1} OM)	Jun-97	ND	ND	ND	ND	ND
	Jul-97	501 (51) A	526 (41) A	502 (32) A	421 (33) AB	328 (26) B
	Aug-97	293 (37) a	247 (20) a	215 (17) ab	197 (17) ab	164 (6) b
	Sep-97	304 (19) a	248 (18) ab	256 (13) ab	243 (29)ab	176 (14) b
	Jul-98	390 (23) A	470 (49) A	468 (39) A	396 (35) A	366 (33) A
Initial NH_4–N (mg kg^{-1} OM)	Jun-97	304 (36) a	190 (29) ab	188 (36) ab	134 (23)	142 (10) b
	Jul-97	270 (25) A	211 (38) AB	143 (31) BC	85 (14) b	116 (34) BC
	Aug-97	158 (38) a	46 (7) ab	27 (4) bc	22 (4) bc	18 (3) c
	Sep-97	141 (19) a	38 (7) ab	32 (4) b	33 (8) b	15 (2) b
	Jul-98	96 (16) A	108 (35) A	106 (18) A	61 (12) A	96 (5) A
Initial (NO_2 + NO_3)–N (mg kg^{-1} OM)	Jun-97	40 (12) a	0 (0) b	0 (0) b	0 (0) b	0 (0) b
	Jul-97	48 (13) a	0 (0) b	0 (0) b	0 (0) b	0 (0) b
	Aug-97	39 (11) a	0 (0) b	0 (0) b	0 (0) b	0 (0) b
	Sep-97	23 (7) a	0 (0) b	0 (0) b	0 (0) b	0 (0) b
	Jul-98	28 (8) a	0 (0) b	0 (0) b	0 (0) b	0 (0) b
Initial DON (mg kg^{-1} OM)	Jun-97	ND	ND	ND	ND	ND
	Jul-97	182 (37) c	315 (8) abc	359 (10) a	336 (24) ab	211 (36) bc
	Aug-97	97 (9) C	202 (16) A	189 (14) AB	175 (15) AB	145 (9) BC
	Sep-97	141 (23) B	211 (12) A	224 (11) A	210 (22) A	161 (12) AB
	Jul-98	266 (18) A	362 (31) A	362 (30) A	334 (26) A	269 (31) A

between net and gross N mineralisation and other organic layer properties were calculated.

Linear mixed model analysis (PROC MIXED procedure SAS 6.12 software package) was used to investigate the degree to which certain organic layer properties accounted for differences in net N mineralisation between the forest sites, and between the incubations in the laboratory (alder/rowan site excluded). The incubations were treated as repeated measures. Net N mineralisation and the initial concentration of NH_4–N were square root transformed in order to obtain equal variances among the groups.

3. Results

3.1. Moisture content and chemical properties of the organic layer

The organic layer was the driest in Aug-97 in all stages except in spruce II, which showed fairly stable field moisture during the whole experiment (Table 3). Comparison of initial and final moisture contents of the field incubated samples (Table 3) indicated that, although the organic layer cores were placed in sealed tubes to eliminate rainwater inputs, the samples received moisture during incubation, especially during the Aug-97 incubation. This might have been due to rainwater entering through the small aeration holes in the upper part of the tubes and also due to condensation of water vapour. However, any N inputs due to the entry of rainwater during the incubations will have been negligible because the throughfall deposition of total N on the spruce II plot was low (<2.5 kg ha^{-1} y^{-1}; Lundgren et al., 1996).

The pH($CaCl_2$) and pH(H_2O) of the organic layer decreased and the C/N ratio increased along the transect from the youngest to the oldest site (Table 1). The TDN and NH_4–N concentrations also decreased correspondingly (Table 3). The alder/rowan site was the only site with detectable concentrations of (NO_2 + NO_3)–N. The DON concentrations were the lowest in the alder/rowan site, followed by the spruce II site.

In the net N mineralisation experiments, both the reference

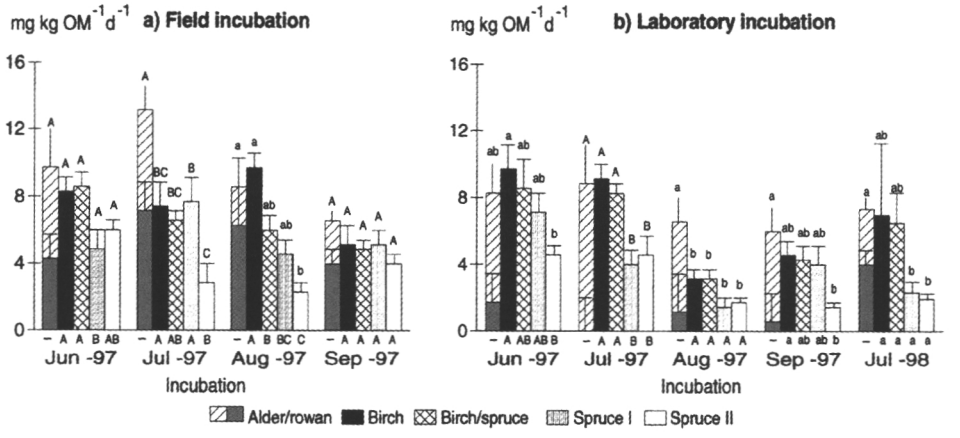


Fig. 3. Daily net N mineralisation during 5-week incubations (a) in the field and (b) in the laboratory. The proportions of NH_4–N (all sites) and (NO_2 + NO_3)–N (slashed bars; the alder/rowan site only) are indicated by solid and hatched bars, respectively. The pairwise comparisons between the forest sites in each incubation are indicated above the bars. The alder/rowan site is excluded from the similar comparisons indicated below the bars. Different capital letters indicate significant differences between the groups according to Tukey's test (rejection level 0.05). Small letters are used in comparisons of the mean ranks. Error bars depict SEM ($n = 8$).

(initial) and incubated (final) organic layer samples were frozen before extraction. Test extraction of both fresh and frozen samples showed that freezing the samples before extraction resulted, on an average, in 3.5- and 1.4-fold increase in NH_4–N and DON concentrations, respectively. However, the slope of the regression between fresh and frozen samples was 1.06 and 1.03 for NH_4–N and for DON concentrations, respectively, and the correlation coefficients were highly significant ($r \approx 0.8$, $P < 0.001$, $n = 40$ for both NH_4–N and DON). The (NO_2 + NO_3)–N concentrations were not affected by freezing. Because the concentrations of NH_4–N, DON and (NO_2 + NO_3)–N in fresh samples were highly correlated with those of the frozen ones, the concentrations of the frozen samples were regarded as valid for further analysis.

3.2. Net N mineralisation and nitrification

In the field incubations, the net N mineralisation tended to decrease along the primary succession transect (Fig. 3a). Net nitrification was observed only in the alder/rowan site.

The sum of the net N mineralisation per unit area for the four field incubations (3-Jun–21-Oct 1997) was 7.4, 7.4, 7.0, 3.6 and 2.8 g m^{-2} in the alder/rowan, birch, birch/spruce, spruce I and spruce II sites, respectively. It was significantly lower in spruce sites I and II than in the other sites (Tukey's test, rejection level 0.05).

The results from the laboratory incubation experiments also showed that net N mineralisation decreased along the successional transect (Fig. 3b). Net N mineralisation rate was the lowest in Aug-97 ($F = 56.09$, $P < 0.001$, df = 1) (Fig. 3b). As in the field incubation experiments, the alder/rowan site was the only site to show net nitrification and there was no significant seasonal variation. The average N mineralisation coefficient (net N mineralisation during Jul-98 laboratory incubation: total N) was 0.008, 0.009, 0.010, 0.005 and 0.005, in alder/rowan, birch, birch/spruce, spruce I and spruce II stages, respectively. The average N mineralisation coefficient thus tended to show lower values in the spruce sites, but the differences between the sites were only tentative ($X^2 = 8.62$, $P = 0.071$).

There was no significant difference in the level of net N mineralisation between the field and laboratory incubations in Jun-97 and in Jul-97. In contrast, the field incubation showed significantly higher net N mineralisation than the laboratory incubation in Aug-97 (paired t-test, $t = 2.65$, $P = 0.012$, df = 39) as well as in Sep-97 ($t = 3.061$, $P = 0.004$, df = 39). The net N mineralisation in the laboratory and that in the field were significantly correlated, with the exception of the incubations in Jun-97 (all forest sites included, Fig. 4a–d).

The net nitrification in the alder/rowan site, the only site where it was observed, was greater in the laboratory than in the field incubations ($F = 8.12$, $P = 0.008$), and the results were significantly correlated only in Jul-97 (Fig. 5; $r_S = 0.71$, $P = 0.047$, $n = 8$).

3.3. Net N mineralisation vs. organic layer properties

As the alder/rowan site differed from the others in relation to nitrification, the results from this plot were treated separately from the other plots. In the combined dataset of the four laboratory incubations, the net nitrification correlated positively ($P < 0.05$) with pH($CaCl_2$) in the range 3.32–4.84, and tentatively with pH(H_2O) (range 4.46–6.04, $P = 0.061$). It also correlated positively with the initial

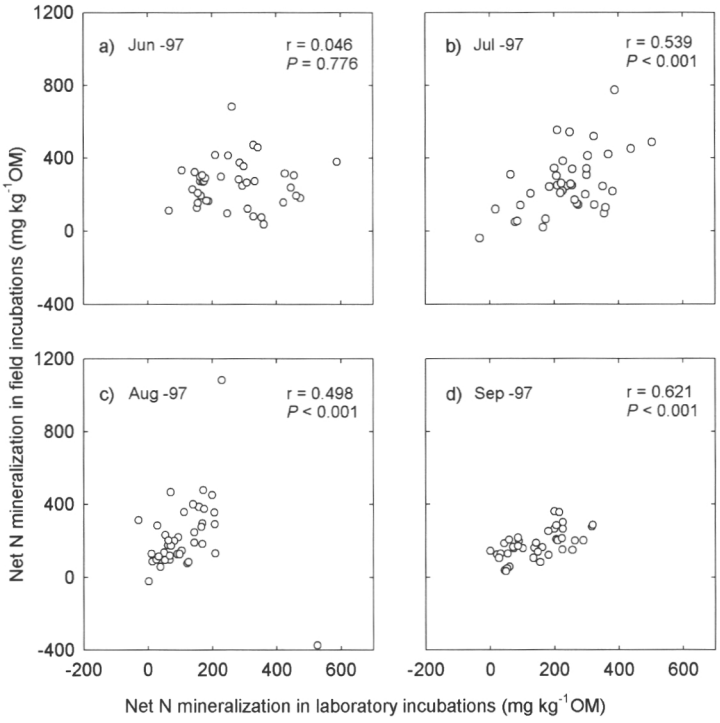


Fig. 4. (a–d) Relationship between net N mineralisation in the laboratory and in the field incubations in (a) Jun-97, (b) Jul-97, (c) Aug-97, and (d) Sep-97. Results are from the alder/rowan, birch, birch/spruce, spruce I and spruce II sites ($n = 40$ in each incubation). In Aug-97 two outliers were omitted from the calculation of the correlation coefficient.

concentrations of TDN, mineral N and $(NO_2 + NO_3)$–N, but not with that of NH_4–N. Net nitrification and net N mineralisation were not significantly correlated. Net N mineralisation correlated positively ($P < 0.05$) with the initial concentrations of TDN, mineral N and NH_4–N.

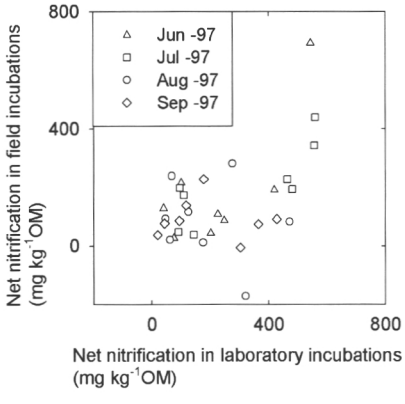


Fig. 5. Relationship between net nitrification in the laboratory and in the field incubations in the alder/rowan site ($n = 8$ in each incubation).

In the laboratory incubations of organic layer samples from the plots other than the alder/rowan site, the net N mineralisation in most cases correlated positively ($P < 0.05$) with pH($CaCl_2$), and with the initial concentrations of TDN, NH_4–N, and DON. In order to investigate the degree to which these variables accounted for differences in net N mineralisation between forest sites, and between the incubations, they were used as covariates, alone or in different combinations, in the statistical model. The effects of significant covariates on the pairwise differences between forest sites and between incubations were compared to the corresponding model without a covariate.

Without a covariate, net N mineralisation showed significant differences between the forest sites and between the incubations (Model I, Table 4). The interaction forest site × incubation was not significant ($P = 0.08$, test not shown). Pairwise comparisons of the least squares means showed that both the birch and birch/spruce sites differed significantly from spruce sites I and II (Model I, Table 5). All the incubations differed significantly from each other, except for those in Jun-97 and Jul-97. However, the significant difference between incubations of Aug-97 and Sep-97 is doubtful because, unlike the other sites, spruce II showed no increase from Aug-97 to Sep-97 (Fig. 3b). This indicates

Table 4
F statistics for the effects of forest site and incubation on net N mineralisation in the laboratory (Model I). In Model II, initial concentrations of NH_4–N and DON were added to the model as covariates. The dependent variable is net N mineralisation in those forest sites where net nitrification was absent (birch, birch/spruce, spruce I, spruce II). Incubation was treated as a repeated measure. Results of the Jun-97 incubation were excluded from Model II because of missing initial DON concentrations

Effect	Covariates	
	Model I (none)	Model II (initial NH_4–N and initial DON)
Covariates		
Initial NH_4–N		
df	–	1
F	–	14.68
P	–	< 0.001
Initial DON		
df	–	1
F	–	27.68
P	–	< 0.001
Forest site		
df	3	3
F	9.74	1.67
P	< 0.001	0.054
Incubation		
df	3	2
F	46.59	3.11
P	< 0.001	0.194

Table 5
Tukey–Kramer adjusted *P* values for pairwise comparisons of least squares means between forest sites and between incubations in the models presented in Table 3

	Model I	Model II
Forest site		
Birch vs. birch/spruce	NS	NS
Birch vs. spruce I	0.0050	NS
Birch vs. spruce II	0.0009	NS
Birch/spruce vs. spruce I	0.0174	NS
Birch/spruce vs. spruce II	0.0032	NS
Spruce I vs. spruce II	NS	NS
Incubation		
Jun-97 vs. Jul-97	NS	–
Jun-97 vs. Aug-97	< 0.0001	–
Jun-97 vs. Sep-97	< 0.0001	–
Jul-97 vs. Aug-97	< 0.0001	NS
Jul-97 vs. Sep-97	0.0002	NS
Aug-97 vs. Sep-97	0.0240	NS

interaction between forest site and incubation, which however, was not statistically significant presumably because of the small number of observations in comparison to the variation in the dataset. pH($CaCl_2$), the initial concentrations of NH_4–N and DON, and the combination of the initial concentrations of NH_4–N and DON, were all significant as covariates (results not shown). The most effective combination of covariates was the initial concentration of NH_4–N and that of DON (Model II, Table 4). For this combination, all pairwise differences between forest sites and between incubations were non-significant (Model II, Table 5). The results of the Jun-97 incubation were excluded from Model II because of missing initial DON concentrations.

3.4. Gross N mineralisation and microbial biomass N

In contrast to the declining trend of net N mineralisation rate along the transect (Fig. 3b), the average gross N mineralisation rate showed a slight increasing trend, although differences between the forest sites were not significant (Fig. 6a). The mean and median of gross:net N mineralisation ratio for all the five forest sites were 8.5 and 5.6, respectively. The recovery of $^{15}NH_4$ was 67.4 ± 2.36%, 50.3 ± 5.39%, 61.3 ± 5.27%, 43.0 ± 5.14% and 58.7 ± 1.94% in the alder/rowan, birch, birch/spruce, spruce I and spruce II sites, respectively. Gross N mineralisation was positively correlated with the initial concentration of DON and TDN and with microbial biomass N, but not with the net N mineralisation nor with the initial concentration of inorganic N (Table 6).

Microbial biomass N per OM was the lowest in the alder/rowan site and differed significantly from that in spruce I (Fig. 6b). The proportion of microbial biomass N out of total N in the organic layer was 2.4, 4.1, 4.1, 6.7 and 5.7% in the alder/rowan, birch, birch/spruce, spruce I and spruce II sites, respectively. Microbial biomass N was positively correlated with the initial concentrations of DON and TDN, but not with that of inorganic N. The correlation between microbial biomass N and C/N ratio in the organic layer was also non-significant. The response of microbial N to the C/N ratio was curvilinear, with the lowest microbial biomass N at the lowest (alder/rowan) and the highest (spruce II) C/N ratios.

4. Discussion

In both the field and laboratory incubations, net N mineralisation decreased along the primary succession transect (alder/rowan, birch, birch/spruce, spruce I and II). The results resemble those obtained in successional floodplain soils along the Tanana River, in interior Alaska. In this area, net ammonification and nitrification were at their highest in the middle successional poplar–alder forest floor, while in the late successional white spruce forests they were at their lowest or even undetectable level (Klingensmith and Van Cleve, 1993). A decrease in net ammonification and nitrification during the course of succession has also been reported in a number of other studies (Robertson, 1982). The transect studied here-alder-rowan, birch, birch-spruce, spruce I and spruce II-is clearly a sequence ranging from a N-rich ecosystem characterised by easily degradable litter and low canopy interception to a N-poor ecosystem with highly recalcitrant litter and high

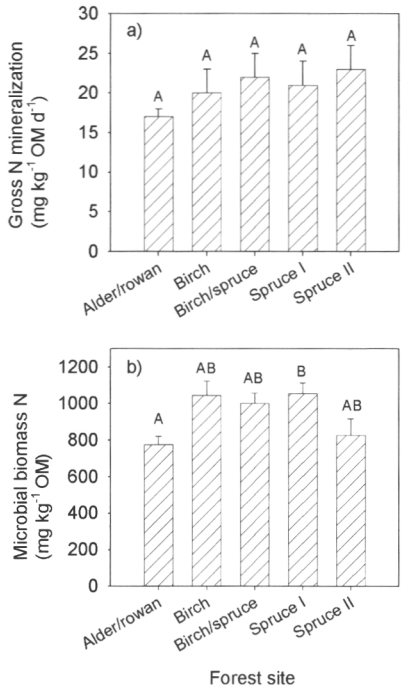


Fig. 6. (a) Gross N mineralisation and (b) microbial biomass N in the forest sites. Error bars depict SEM ($n = 7$–8).

canopy interception. Our estimates for the net N mineralisation on an areal basis (2.8–7.4 g m^{-2}) are fairly consistent with those reported in Norway spruce stands in Sweden and Denmark (1.7–6.8 g m^{-2} y^{-1} in the LFH layer; Persson et al. (1995)).

In contrast to decreasing net N mineralisation rates, gross mineralisation of N showed a tentative increase along the transect, although differences between the forest sites were non-significant. Similarly, in a study concerning net and gross N mineralisation below birch (*Betula papyrifera* Marsh.), spruce (*Picea glauca* (Moench) A. Voss) and alder (*Alnus incana* (L.) Moench) in Isle Royale, Michigan, alder forest showed the highest net N mineralisation rate, but gross mineralisation was the highest beneath spruce and birch (Stottlemyer and Toczydlowski, 1999). The authors concluded that higher net N mineralisation rates beneath alder in comparison to birch and spruce resulted from lower microbial immobilisation rather than greater gross N mineralisation. In our study microbial biomass N was not followed during the incubation, but increasing microbial biomass N along the transect from alder/rowan to spruce I in Jul-98 in part supports this interpretation. However, the stable or even increasing gross N mineralisation rate observed along the transect appears to be inconsistent with the fact that N availability and soil organic matter decomposability, as revealed by the increasing C/N ratio in the organic layer, concurrently and distinctively decreased. This contradiction in the results may be explained by the nature of the $^{15}NH_4^+$ pool dilution method used, as discussed in the recent paper of Fierer et al. (2001). The proportion of microbial biomass N out of total N in the organic layer increased along the transect and hence the rate of gross N mineralisation per unit of microbial biomass N remained fairly stable. Thus, the gross mineralisation rates observed would appear plausible, if pool dilution measured the microbial cycling and recycling of small pools of highly labile, N-rich compounds rather than the overall decomposition of soil organic matter and microbial growth, as suggested by Fierer et al. (2001).

Net N mineralisation was related to both the total (as Munson and Timmer (1991)) and dissolved N concentrations. The N mineralisation coefficient (Weier and MacRae, 1993), i.e. net N mineralisation as a proportion of the total N concentration of the organic layer, tentatively declined in

Table 6
Spearman rank correlation coefficients and their P values ($n = 37$–40) between gross and net N mineralisation, microbial biomass N, inorganic, organic (DON) and total dissolved (TDN) nitrogen concentrations in the beginning of the incubation experiments, and C/N ratio and pH($CaCl_2$) of the organic layer. NS = not significant

	Gross N mineralisation	Net N mineralisation	Microbial biomass N	Initial inorganic N	Initial DON	Initial TDN	C/N ratio
Net N mineralisation	NS						
Microbial biomass N	0.358 0.030	NS					
Initial inorganic N	NS	0.563 < 0.001	NS				
Initial DON	0.411 0.009	NS	0.641 < 0.001	NS			
Initial TDN	0.371 0.020	0.308 0.053	0.568 < 0.001	0.504 < 0.001	0.893 < 0.001		
C/N ratio	NS	−0.605 < 0.001	NS	−0.332 0.036	NS	−0.308 0.054	
pH($CaCl_2$)	NS	0.588 < 0.001	NS	0.512 < 0.001	NS	NS	−0.572 < 0.001

the spruce sites compared to the sites representing earlier successional stages. This is assumed to be an indication of the more recalcitrant nature of soil organic matter, generally expressed as higher lignin content and C/N ratio (Berg, 1986) in late successional spruce forests than in the preceding deciduous stands (Pastor et al., 1987; Priha and Smolander, 1999; Côte et al., 2000).

Microbial biomass N tended to increase along the transect from the alder/rowan site to spruce I, but was lower again in spruce II. Correspondingly, since the C/N ratio increased along the transect, the response of microbial biomass N to the C/N ratio was curvilinear rather than linear. This may indicate that the microbes in the N-rich alder/rowan site are relatively C limited, as reported previously for early successional alder stage by Clein and Schimel (1995). During the succession, reducing N pool leads to N limitation of the microbial community (Ohtonen et al., 1992; Aikio et al., 2000). In the spruce II site, microbial growth may again become limited by factors other than nitrogen, e.g. by more recalcitrant C sources.

Net nitrification only occurred in the alder/rowan site. This result is consistent with those of Van Cleve et al. (1993) and Hart and Gunther (1989). The latter authors found net nitrification in the soil only in the alder site when four different subarctic vegetation types (alder, dry tundra, moist tundra and white spruce sites) were compared. However, a lack of net nitrification does not necessarily indicate the absence of nitrifiers, because the nitrate formed may be rapidly immobilised by soil micro-organisms (Stark and Hart, 1997; Stottlemyer and Toczydlowski, 1999). Dinitrogen-fixing alder influences mineral soil N transformations both by increasing the size of the total N pool in the soil, and by supplying higher quality litter inputs to the forest floor than non-dinitrogen-fixing plants (Hart and Gunther, 1989). In our study, the organic layer of the alder/rowan site differed from that of later successional sites in having a higher pH and greater N availability. Plant secondary compounds may also have played a role in the cessation of net nitrification after the transition from a dominance of alder and rowan to birch and further to spruce. In Alaskan river floodplains, for instance, the rapid decrease in N_2 fixation and nitrification during the transition from alder to balsam poplar has been attributed to the effects of secondary compounds in balsam poplar on microbial activity (Schimel et al., 1996, 1998; Fierer et al., 2001).

Within the alder/rowan site, net nitrification was found to correlate positively with pH($CaCl_2$) in the range of 3.32–4.84, which is in accordance with the results of other studies (Smolander et al., 1998; Ste-Marie and Paré, 1999). Net nitrification was also positively correlated with the initial concentrations of TDN, mineral N and (NO_2 + NO_3)–N, but not with that of NH_4–N. Although NH_4–N is the substrate for autotrophic nitrifying bacteria, the initial concentration of (NO_2 + NO_3)–N, which is the net product of recent nitrification activity, predicted the rate of net nitrification and the actual substrate supply better than the initial concentration of NH_4–N.

The clearest seasonal variation in net N mineralisation rate was attributed to the significant decrease in this rate in laboratory incubations of Aug-97 compared to the incubations of Jun-97 and Jul-97. According to observations from the nearest meteorological station in Vaasa (data not shown), the sampling day in Aug-97 was preceded by an almost rainless 2-week period, and, consequently, the moisture content of the samples at the time of sampling was low. Thus, soil moisture, generally regarded as a major factor controlling the activities of microbes and microfauna (Schlentner and Van Cleve, 1985; Wagener and Schimel, 1998), might have reduced the microbial populations and activity in the field. This conclusion is supported by the fact that products of microbial activity in the near past, i.e. initial concentrations of NH_4–N and DON in samples, also declined in Aug-97 and accounted for the differences in net N mineralisation between the incubation periods in the laboratory (alder/rowan site excluded). Although the moisture content was kept constant during the actual incubation period in the laboratory, the preceding drought period may have resulted in a depression in long-term microbial activity. This interpretation is consistent with those of Clein and Schimel (1994) and Schimel et al. (1999). Based on the experiments on decomposing birch litter, they concluded that drying and rewetting events had a strong and prolonged effect on the size and activity of the litter microbial community in laboratory conditions.

In the field incubations, net N mineralisation showed no clear seasonality nor covariation with initial concentrations of NH_4–N and DON. The similar decrease in the net N mineralisation rate as in Aug-97 in the laboratory was thus not observed in the field. The post-incubation moisture contents in the field-incubated samples indicated that, although the samples were kept in sealed tubes, they appear to have become moistened during incubation, either as a result of rainwater passing through the small aeration holes in the upper part of the tubes, or from condensation. If we assume that the decrease in the net N mineralisation in the laboratory was due to the long-term effects of drought on microbial populations, then this result suggests that recovery and recolonization of the microbial community occurred more rapidly in intact soil cores than in sieved and homogenized samples in the laboratory. In spite of the difference in the net N mineralisation rate between the field and laboratory incubations in Aug-97, these two methods gave fairly similar results, and for instance the effect of temperature fluctuations in the field was not reflected in the rates of N mineralisation. The methodological differences became apparent in the net nitrification rates for the alder/rowan site, which were higher in the laboratory than in the field incubations.

For the first time in the land-uplift area along the coast of the Gulf of Bothnia, this study presented the changes in N transformations along a primary succession transect,

beginning from an early successional N-rich alder/rowan site with easily degradable litter inputs, proceeding to N-deficient spruce sites with poor soil organic matter quality. Despite differences e.g. in plant species composition and soil acidity, our results fit well to comparable studies from the boreal region of Alaska. As such, our results basically confirm the conclusions of Alaskan studies that declining nitrogen availability, and hence productivity during successional development of forest ecosystems, are largely related to changes in organic matter quality through the control of microbial activity. Low N availability may also contribute to the poor crown condition (Merilä et al., 1998) and growth (Karlsson, 2000) of the coastal Norway spruce forests in western Finland.

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Paper IV

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IV

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Soil microbial activity and community structure along a primary succession transect on the land-uplift coast in western Finland

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Abstract

We investigated the changes in basal respiration (BASAL), microbial biomass as substrate-induced respiration (SIR) and their ratio (qCO_2) in the organic layer along a primary successional transect (alder/rowan, birch, birch/spruce, spruce I and spruce II) typical of the land-uplift coast in western Finland. PLFA analysis was used to detect concurrent successional changes in microbial community structure. Along the transect the soil C/N ratio increased (from 16 to 37) and pH (H_2O) decreased (from 5.11 to 3.98) substantially. Concurrently, BASAL and SIR remained mainly stable although, during the most favorable temperature and moisture conditions in the field, they tended to increase along the transect from the alder/rowan site to spruce I, and decreased again in spruce II. qCO_2 showed no consistent trend along the transect. The soil of the birch site had the highest total PLFA and bacterial PLFA concentrations, while the soils of both spruce sites had the lowest. The ordination configuration of the PLFA data in non-metric multidimensional scaling was clearly related to the C/N ratio and pH, and separated the forest sites relatively well. It was possible, on the basis of the similarities in the variation pattern along the transect, to divide the PLFAs into six groups.

Keywords: *Alnus incana*; *Betula* sp; Forest soil; Phospholipid fatty acid; *Picea abies*; Soil respiration; Substrate-induced respiration

1. Introduction

Succession of soil microbial communities proceeds interdependently with the development of the forest vegetation, and is controlled by the prevailing climatic and edaphic conditions. Forest vegetation affects the environmental conditions and energy supply of soil microbes through the functions of the roots, above- and below-ground litter production, and interception and leaching processes in the canopies, all of which change during succession. Microbes play a key role in the decomposition and mineralisation of dead organic matter, and thus in the cycling of carbon and nutrients. In addition, decomposition processes result in the formation of recalcitrant organic compounds, which subsequently leads to the formation of an organic layer on the soil surface.

Postglacial land-uplift in the coastal area of the Gulf of Bothnia (8–9 mm yr^{-1}; Mäkinen et al., 1986) offers an opportunity to follow successional changes of the forest ecosystem along a spatial continuum. On till soils the succession of forest vegetation begins from a deciduous stage dominated by N_2-fixing alder (*Alnus incana* L.) and proceeds to Norway spruce (*Picea abies* (L.) Karst.) stands, often via a birch (mainly *Betula pubescens* Ehrh.) dominated forest stage (Appelroth, 1948; Ericson and Wallentinus, 1979). The organic layer properties were found to change drastically along a primary successional transect in this area (Merilä et al., 2002). The C/N ratio, frequently used as a predictor of the decomposability of soil organic matter, increased from an average of 16 in the early successional alder/rowan site to 37 in the spruce site. A concurrent decrease in pH (H_2O) (from 5.11 to 3.98) and net N mineralisation were apparent. Similar changes in soil organic matter quality have often been found to occur in other areas during primary succession, with substantial effects on soil microbial activity (Crocker and Major, 1955; Van Cleve et al., 1993) and community structure (Pennanen et al., 2001).

Based on the C/N ratio of heterotrophic microbial cells and losses of C due to respiration, a ratio of 30:1 has been

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proposed as the critical C/N ratio for detritus, above which heterotrophic micro-organisms are N limited and below which they are C limited (Tate, 1995). From this it can be inferred that the microbes in the N-rich alder/rowan stage along the transect may be relatively the most C limited, resulting in lower microbial biomass and activity in comparison to the subsequent forest sites (Clein and Schimel, 1995; Merilä et al., 2002). At some stages of succession, a reduction in the N-pool might lead to N limitation of the microbial community. In the late successional spruce sites recalcitrant C sources may also result in reduced microbial biomass and activity, and affect community composition (Flanagan and Van Cleve, 1983; Mikola, 1985; Bradley and Fyles, 1995; Priha and Smolander, 1999; Saetre et al., 1999; Hobbie et al., 2000; Priha et al., 2001). Consequently, we hypothesized that the response of basal respiration and microbial biomass to substrate quality along the transect would be concavely curvilinear, with the lowest basal respiration and microbial biomass occurring in the early successional alder/rowan site and the late successional spruce site. We also aimed to evaluate the hypothesis proposed by Insam and Haselwandter (1989), according to which the carbon-use efficiency of the soil microbial community increases during ecosystem succession, a higher proportion of carbon being allocated into the biomass, resulting in a decrease in the respiration to biomass ratio of soil microbes (qCO_2). This hypothesis has been supported by Ohtonen et al. (1999) but criticized by Wardle and Ghani (1995). The latter authors emphasized that qCO_2 does not decline predictably in response to ecosystem development whenever stress (e.g. nutrient limitation, increased acidity) increases along successional gradients. Nevertheless, the structure of the microbial community has been found to change along successional transects and, as a consequence, changes in microbial activities have been observed (Ohtonen et al., 1999; Pennanen et al., 2001).

With the earlier mentioned hypothesis in mind, the objective of our study was to investigate the changes in microbial respiration, biomass and their ratio along a primary successional transect typical for the land-uplift coast of the Gulf of Bothnia. PLFA analysis (Tunlid and White, 1990) was used to detect concurrent changes in the microbial community structure. Seasonal fluctuation in these variables was also analyzed.

2. Material and methods

2.1. Study area, sampling and analyses

The study was conducted along a primary successional transect in the archipelago of Raippaluoto in western Finland (63°20′N, 21°15′E). The transect comprised the following four forest sites: alder/rowan (70 year-old *A. incana* (L.) Moench/*Sorbus aucuparia* L.), birch (mainly 80 year-old *B. pubescens* Ehrh.), birch/spruce (75 year old *B. pubescens* Ehrh. and *B. pendula* Roth/*P. abies* (L.) Karst.) and spruce I (95 year-old *P. abies*). In order to extend the age sequence, a fifth forest site (spruce II; 130 year old *P. abies*) was chosen 12.2 km to the south of the transect. The height and approximate years above sea level, and some organic layer characteristics of each plot, are presented in Table 1. The sites are described in detail in Merilä et al. (2002).

This study focuses on the organic soil layer, also shortened to 'soil' throughout the paper. The soils sampled for the study are the same as those described in Merilä et al. (2002). In brief, samples for chemical and microbial analyses were taken four times in 1997: 3 June, 8 July, 11 August, 16 September, here named Jun-97, Jul-97, Aug-97, Sep-97, respectively, and once again in 13–14 July 1998 (Jul-98). Twenty-four soil cores were sampled systematically at each plot, any visible mineral soil, if present, being carefully removed. The soil samples from three adjacent cores were combined to give eight samples per plot. For the biological analyses, the samples were kept cool (4 °C) until sieved and weighed. Moisture (105 °C, 24 h), loss in weight on ignition (OM; 500 °C, 4 h), pH (sample/water 1:3 v/v, measurement after leaving to stand overnight) and in Jul-98 total C and N (LECO CHN-600) of the soil samples were determined. The samples were kept frozen until further analyzed.

The samples were moistened to a water content that equaled 250% of the OM content, and three replicates were analyzed for microbial variables using a respirometer (Nordgren, 1988). After the beginning of the experimental run, basal respiration (BASAL) was analyzed for at least 40 h at a stable rate of respiration, after which 200 mg of glucose, 22 mg of N as $(NH_4)_2SO_4$ and 2.4 mg of P as KH_2PO_4 g^{-1} OM of sample were added with vigorous shaking of the sample in order to determine the substrate-induced respiration (SIR), which is correlated to microbial biomass (Anderson and Domsch, 1978). This was calculated as the average CO_2 evolution over a 5 h period starting 3–6 h after addition of the substrate. Lag-time (Lag) of the microbial community, which is the time period from substrate addition to the start of growth of the microbial community, was finally ascertained. qCO_2 was calculated as the BASAL/SIR ratio.

Phospholipid fatty acid (PLFA) patterns were determined for each forest site from one composite sample of each sampling in 1997 (4×5 samples) by extracting and analyzing as described by Pennanen et al. (1999). Briefly, 0.5 g fresh weight of soil was extracted with a chloroform/methanol/citrate buffer mixture (1:2:0.8) and the lipids separated into neutral lipids, glycolipids and phospholipids on a silicic acid column. The phospholipids were subjected to a mild alkaline methanolysis, and the fatty acid methyl esters were detected by gas chromatography (flame ionization detector) using a 50-M HP-5 (phenylmethyl silicone) capillary column and helium as carrier gas. Peak

Table 1
The height and approximate years above sea level for each plot and thickness, loss in weight on ignition, pH (H_2O), C/N ratio and total N and extractable nutrients (Halonen et al., 1983) of the organic layer of the successional forest sites studied. For further information see Merilä et al. (2002)

	Alder/rowan	Birch	Birch/spruce	Spruce I	Spruce II
Meters above see level	0.8	2.1	2.3	4.2	8.3
Approximate years above sea level	90	250	270	500	980
Organic layer					
Thickness (cm)	6.5	6.6	7.4	6.6	6.8
Loss in weight on ignition (OM) (%)	87.2	87.0	90.2	84.9	84.7
pH (H_2O)	5.11	4.32	4.02	4.06	3.98
C/N ratio	15.9	20.2	21.4	31.7	37.3
Total N (mg g^{-1} OM)	33.0	25.7	25.1	16.0	14.3
NH_4-Ac (pH 4.65) ext. nutrients (mg kg^{-1} OM)					
P	200	240	160	350	200
S	150	180	200	160	140
K	840	850	750	880	650
Ca	3010	1900	1150	1980	2450
Mg	1920	760	480	550	480

areas were quantified by adding methyl non-adecanoate fatty acid (19:0) as an internal standard.

Total microbial biomass (TotPLFA) was determined as the sum of all the extracted PLFAs. The sum of PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0,17:0, cy17:0, 18:1ω7 and cy19:0) was used as an index of the bacterial biomass (BactPLFA) (Frostegård and Bååth, 1996). The quantity of 18:2ω6 was used as an indicator of fungal biomass (FungPLFA), since 18:2ω6 in soil is known to be of mainly fungal origin (Federle, 1986) and it is known to correlate with the amount of ergosterol (Frostegård and Bååth, 1996), a compound found only in fungi. The ratio of FungPLFA/BactPLFA was used as an index of the ratio of fungal/bacterial biomass in the soil.

2.2. Statistical analysis

Because this study was conducted on a single transect and successional stages were thus not replicated, statistical comparisons could be made to distinguish differences between sites on the transect but not between the successional stages.

Differences in the variables between the sites and between the samplings in 1997 were tested with repeated measures analyses of a general linear model. In the case that the sphericity assumption of univariate repeated measures ANOVA was not violated according to Mauchly's test (Crowder and Hand, 1990), contrasts were used to test the differences among variables between subsequent samplings (SPSS® version 9.0.1). The non-parametric Kruskal–Wallis test was used to distinguish differences between the sites, and pairwise differences were tested with Tukey's test. In the case that the equality of variances between the groups was not met, pairwise comparisons were made for mean ranks.

The PLFA data were ordered by non-metric multidimensional scaling (NMS) by PC-ORD software 4.14 (autopilot mode with slow and thorough iteration; McCune and Mefford, 1999). Prior to NMS, the mole percentages of the PLFA values were double-square root transformed ($y^{0.25}$) to down-weight the influence of the very abundant PLFAs. Sørensen (Bray & Curtis) distance was applied as a measure of dissimilarity in microbial community structure between the samples. C/N ratio and pH in the soil, BactPLFA, TotPLFA and the FungPLFA/BactPLFA ratio were given as vectors in the ordination graph, the direction of each arrow indicating the direction of the gradient and the length indicating the strength of correlation. The final configuration was rotated by pH.

3. Results

3.1. Microbial activity and biomass

BASAL in Jul-97 and BASAL and SIR in Jul-98 tended to increase along the transect from the alder/rowan to spruce I and to decrease again in spruce II (Table 2). In Sep-97 spruce I showed the highest BASAL and SIR rates, while spruce II showed the lowest SIR. Otherwise there were no significant differences in BASAL or in SIR between the forest sites.

The five forest sites showed no uniform seasonal pattern in BASAL, SIR, qCO_2 or Lag. However, in the birch/spruce, spruce I and spruce II sites BASAL and SIR increased from Jun-97 to Jul-97 ($p < 0.001$); this also occurred in SIR in the birch site. Further, in the birch/spruce and spruce I sites BASAL and SIR ($p < 0.05$) decreased from Jul-97 to Aug-97, and again this also occurred in SIR in the birch site. Finally, SIR showed an increase from Aug-97 to Sep-97 ($p = 0.001$) in the birch, birch/spruce and spruce I sites.

Table 2
Mean ± S.E. ($n = 6–8$) for basal respiration (BASAL), substrate induced respiration (SIR), BASAL: SIR ratio (qCO_2), and lag time (Lag) and Mean ± S.D. ($n = 4$) for total, bacterial and fungal PLFAs and ratio of fungal and bacterial PLFAs in the successional forest sites. According to Tukey's test (rejection level 0.05) the sites labeled with the same letter do not differ significantly. Small letters are used for the comparison of mean ranks

	Sampling	Alder/rowan		Birch		Birch/spruce		Spruce I		Spruce II	
Field moisture (g g^{-1} OM)	Jun-97	1.89 ± 0.16	B	2.78 ± 0.22	A	2.80 ± 0.20	A	1.85 ± 0.15	B	1.96 ± 0.20	B
	Jul-97	1.72 ± 0.08	B	2.33 ± 0.10	A	2.15 ± 0.09	A	2.22 ± 0.13	A	2.32 ± 0.11	A
	Aug-97	1.22 ± 0.08	D	1.90 ± 0.16	AB	1.75 ± 0.09	A	1.46 ± 0.09	CD	2.20 ± 0.10	A
	Sep-97	2.31 ± 0.15	A	2.50 ± 0.10	A	2.49 ± 0.14	BC	2.66 ± 0.21	A	2.73 ± 0.14	A
	Jul-98	1.75 ± 0.21	B	2.82 ± 0.21	A	2.57 ± 0.15	A	2.25 ± 0.20	AB	2.54 ± 0.18	A
BASAL (μg CO_2 g^{-1} OM h^{-1})	Jun-97	57 ± 5	A	62 ± 7	A	52 ± 3	A	53 ± 5	A	48 ± 6	A
	Jul-97	46 ± 3	B	65 ± 8	AB	77 ± 6	A	77 ± 10	A	57 ± 8	AB
	Aug-97	63 ± 5	A	61 ± 4	A	61 ± 7	A	64 ± 6	A	67 ± 6	A
	Sep-97	54 ± 5	B	57 ± 4	B	60 ± 5	AB	81 ± 9	A	45 ± 4	B
	Jul-98	67 ± 6	B	85 ± 5	AB	87 ± 6	AB	108 ± 12	A	86 ± 8	AB
SIR (μg CO_2 g^{-1} OM h^{-1})	Jun-97	91 ± 15	A	106 ± 12	A	86 ± 7	A	91 ± 10	A	69 ± 11	A
	Jul-97	103 ± 10	A	135 ± 20	A	114 ± 9	A	142 ± 24	A	108 ± 19	A
	Aug-97	115 ± 13	A	77 ± 5	A	77 ± 6	A	102 ± 11	A	100 ± 8	A
	Sep-97	116 ± 19	ab	115 ± 12	ab	111 ± 4	ab	141 ± 19	a	79 ± 8	b
	Jul-98	94 ± 6	B	122 ± 13	AB	130 ± 9	AB	146 ± 22	A	119 ± 13	AB
qCO_2	Jun-97	0.68 ± 0.08	A	0.60 ± 0.04	A	0.63 ± 0.05	A	0.59 ± 0.03	A	0.74 ± 0.03	A
	Jul-97	0.46 ± 0.03	B	0.50 ± 0.05	B	0.69 ± 0.05	A	0.57 ± 0.05	AB	0.55 ± 0.03	AB
	Aug-97	0.57 ± 0.03	C	0.80 ± 0.03	A	0.78 ± 0.03	AB	0.64 ± 0.04	C	0.67 ± 0.03	BC
	Sep-97	0.50 ± 0.04	A	0.52 ± 0.05	A	0.55 ± 0.05	A	0.61 ± 0.06	A	0.59 ± 0.06	A
	Jul-98	0.72 ± 0.03	A	0.74 ± 0.06	A	0.68 ± 0.02	A	0.76 ± 0.05	A	0.74 ± 0.04	A
Lag time (h)	Jun-97	10.3 ± 1.3	a	8.9 ± 0.6	A	7.8 ± 0.3	a	8.1 ± 0.6	a	10.9 ± 0.8	a
	Jul-97	9.7 ± 0.6	A	6.4 ± 0.6	BC	6.2 ± 0.5	C	8.4 ± 0.4	AB	8.8 ± 0.4	A
	Aug-97	10.6 ± 1.0	A	9.9 ± 0.4	A	8.5 ± 0.5	AB	6.4 ± 0.7	B	8.9 ± 0.3	AB
	Sep-97	18.6 ± 0.6	A	10.7 ± 0.7	B	10.1 ± 1.0	BC	7.4 ± 7.4	C	8.0 ± 0.8	BC
	Jul-98	11.6 ± 0.9	A	8.3 ± 0.4	B	8.6 ± 0.6	B	8.0 ± 0.5	B	8.9 ± 0.6	B
TotPLFA (nmol g^{-1} OM)	Jun–Sep-97	1200 ± 218		1430 ± 134		1220 ± 124		1000 ± 194		1041 ± 110	
BactPLFA (nmol g^{-1} OM)	Jun–Sep-97	490 ± 59		580 ± 48		510 ± 54		380 ± 68		400 ± 36	
FungPLFA (nmol 18:2ω6 g^{-1} OM)	Jun–Sep-97	50 ± 6		64 ± 9		46 ± 5		72 ± 13		47 ± 9	
FungPLFA/BactPLFA	Jun–Sep-97	0.10 ± 0.012		0.11 ± 0.011		0.09 ± 0.008		0.19 ± 0.008		0.12 ± 0.027	

In Jul-97 qCO_2 was significantly higher in the birch/spruce site than in the preceding successional sites ($p = 0.010$). In Aug-97 the highest qCO_2s were observed in the birch and birch/spruce sites. In the other samplings, qCO_2 showed no significant differences between the forest sites. The alder/rowan and spruce II sites showed similar seasonality in qCO_2: decreasing from Jun-97 to Jul-97 ($p < 0.001$), increasing from Jul-97 to Aug-97 ($p < 0.001$), and again decreasing from Aug-97 to Sep-97 ($p = 0.016$).

Apart from the sampling of Jun-97, Lag tended to be longer in the alder/rowan than in the other sites, the difference being significant in Sep-97 ($p < 0.001$) and in Jul-98 ($p = 0.024$).

3.2. Microbial community structure

The soil of the birch site had the highest TotPLFA and BactPLFA concentrations, while in the soil of both spruce sites they were the lowest (Table 2). FungPLFA and the ratio FungPLFA/BactPLFA were at their highest level in spruce I (Table 2).

The ordination configuration of PLFA data in NMS was clearly related to the C/N ratio and pH in the soil, and separated the forest sites along the transect relatively well (Fig. 1(a)). The most distinguishable group was formed by the samples of the alder/rowan site, which showed the highest pH and lowest C/N ratio (Table 1). The samples from the birch and birch/spruce sites were grouped relatively close to each other, but the third group formed by the samples of spruce sites I and II were somewhat more dispersed.

It was possible, on the basis of the similarities in the variation pattern along the transect, to divide the PLFAs into six groups (Table 3), presented also in NMS configuration (Fig. 1(b)). The PLFAs of Group 1 (16:1ω5, cy17:0, 18:1, 18:1ω7, 10Me18:0, 19:1a) were relatively more abundant in the alder/rowan site in comparison to the other sites. Group 2 (i14:0, a15:0, i17:0, br18:0) also showed the highest relative amounts in the alder/rowan site and, in addition, gradually decreased along the transect. Group 3 consisted of seven

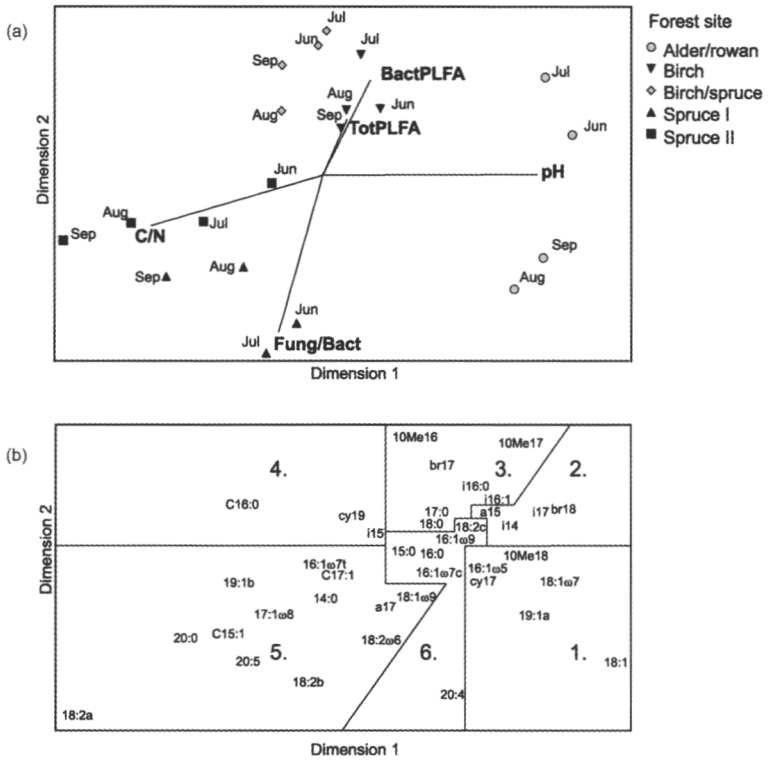

Fig. 1. Two-dimensional solution of NMS. (a) Ordination of the samples and the fitted vectors of soil pH, C/N ratio, bacterial (BactPLFA) and total microbial (TotPLFA) biomass, and ratio between fungal and bacterial biomass (Fung/Bact). June, July, August and September denote the month of sampling in 1997. (b) The weighted averages of the PLFAs. The PLFAs were divided into six groups (1.–6.) on the basis of the similarities in their variation pattern along the transect (see Table 3).

PLFAs (i16:0, i16:1, 10Me16:0, 10Me17:0, 17:0, br17:0, 18:0) which were the most abundant either in the birch site or in the birch/spruce site, and three of them (i16:0, i16:1 and 10Me17:0) were present in minimum amounts in spruce sites I and II. Group 4 (i15:0, cy19:0, c16:0) was characterized by a relatively low abundance in the alder/rowan site compared to the other sites. The feature common to the PLFAs of Group 5 (13 PLFAs, see Table 3) was that each of them showed the highest relative abundance in either of the spruce sites. Group 6 included PLFAs showing stable or inconsistently variable amounts along the transect (Table 3).

Generally, the samples of Jun-97 and Jul-97 (summer) were separated from those of Aug-97 and Sep-97 (autumn) on the basis of their position in NMS configuration, thus showing a slight seasonal dichotomy within each forest site (Fig. 1(a)). This dichotomy was due to the trend of certain PLFAs to increase from summer to autumn samplings. PLFA 20:5 showed this pattern in all sites, the overall means being 0.35 ± 0.03 and 1.02 ± 0.10 mol% in the summer and autumn samplings, respectively. Especially the alder/rowan site, but also the birch site, had lower relative amounts of PLFA 18:1 in summer (0.25 ± 0.02 and 0.50 ± 0.10 mol%, respectively) than in autumn (2.80 ± 0.12 and 0.94 ± 0.22 mol%, respectively). The increase in PLFAs 19:1b and 20:0 from summer to autumn occurred most clearly in both spruce sites (1.09 ± 0.14 and 1.04 ± 0.15 mol% in summer, respectively, and 2.18 ± 0.30 and 2.56 ± 0.62 mol% in autumn, respectively).

3.3. Relationships between the different microbial activity and biomass measurements

SIR showed no significant correlation with TotPLFA in any of the samplings. In contrast, SIR and FungPLFA tended to be correlated, and the correlation was significant ($r_S = 0.900$, $p = 0.037$, $n = 5$) in Jul-97.

In Jul-98 gross N mineralisation rate was measured on the same samples as those used in this study (Merilä et al.,

Table 3
Means and standard deviations ($n = 4$) for amounts (mol%) of PLFAs in the organic layer of primary successional forest sites sampled in 1997

PLFA	Alder/rowan	Birch	Birch/spruce	Spruce I	Spruce II	Total
Maximum in the alder/rowan, otherwise rather stable						
16:1ω5	2.25 ± 0.24	1.84 ± 0.25	1.63 ± 0.19	1.82 ± 0.24	1.67 ± 0.29	1.84 ± 0.31
cy17:0	3.07 ± 0.43	2.22 ± 0.18	2.27 ± 0.22	2.63 ± 0.11	2.02 ± 0.30	2.44 ± 0.45
18:1	1.53 ± 1.47	0.72 ± 0.32	0.32 ± 0.10	0.34 ± 0.09	0.33 ± 0.12	0.65 ± 0.77
18:1ω7	9.32 ± 0.53	5.16 ± 0.57	4.48 ± 0.30	5.04 ± 0.51	4.79 ± 0.47	5.76 ± 1.89
10Me18:0	1.62 ± 0.11	1.23 ± 0.10	0.92 ± 0.16	1.13 ± 0.33	1.00 ± 0.14	1.18 ± 0.30
19:1a	0.76 ± 0.13	0.40 ± 0.05	0.35 ± 0.06	0.53 ± 0.08	0.35 ± 0.06	0.48 ± 0.17
Decreasing along the transect						
i14:0	0.61 ± 0.03	0.49 ± 0.06	0.47 ± 0.05	0.36 ± 0.03	0.41 ± 0.06	0.47 ± 0.10
a15:0	4.24 ± 0.32	3.77 ± 0.25	3.98 ± 0.25	2.83 ± 0.08	3.20 ± 0.36	3.60 ± 0.58
i17:0	1.71 ± 0.23	1.26 ± 0.12	1.25 ± 0.12	0.91 ± 0.08	0.99 ± 0.10	1.22 ± 0.32
br18:0	2.06 ± 0.42	1.18 ± 0.07	1.37 ± 0.07	0.89 ± 0.21	1.11 ± 0.19	1.32 ± 0.46
Maximum in the birch or birch/spruce site						
i16:0	5.94 ± 0.77	6.49 ± 0.53	6.66 ± 0.59	4.59 ± 0.67	4.93 ± 0.30	5.72 ± 1.00
i16:1	1.30 ± 0.18	1.42 ± 0.16	1.22 ± 0.17	0.93 ± 0.23	0.98 ± 0.24	1.17 ± 0.26
10Me17:0	1.68 ± 0.29	2.49 ± 0.33	2.17 ± 0.46	1.15 ± 0.22	1.26 ± 0.12	1.75 ± 0.59
10Me16:0	5.84 ± 1.25	8.64 ± 0.74	10.51 ± 1.33	5.23 ± 2.10	7.52 ± 1.17	7.55 ± 2.32
17:0	0.64 ± 0.02	0.74 ± 0.05	0.83 ± 0.05	0.68 ± 0.02	0.59 ± 0.09	0.70 ± 0.10
br17:0	0.25 ± 0.06	0.35 ± 0.03	0.36 ± 0.02	0.24 ± 0.10	0.25 ± 0.04	0.29 ± 0.07
18:0	2.27 ± 0.11	2.43 ± 0.15	2.70 ± 0.28	2.16 ± 0.12	2.11 ± 0.13	2.33 ± 0.27
Minimum in the alder/rowan, otherwise rather stable						
i15:0	8.15 ± 0.39	10.86 ± 0.29	11.19 ± 0.66	10.53 ± 0.65	10.60 ± 0.61	10.27 ± 1.21
c16:0	0.17 ± 0.02	0.45 ± 0.05	0.56 ± 0.06	0.48 ± 0.09	0.55 ± 0.05	0.44 ± 0.16
cy19:0	3.54 ± 0.70	5.34 ± 0.37	6.14 ± 0.46	5.02 ± 0.50	5.21 ± 0.67	5.05 ± 1.00
Maximum in the spruce site I or II						
14:0	1.23 ± 0.09	1.24 ± 0.07	1.24 ± 0.14	1.72 ± 0.18	2.02 ± 0.38	1.49 ± 0.38
c15:1	0.18 ± 0.13	0.16 ± 0.05	0.14 ± 0.04	0.21 ± 0.11	0.41 ± 0.11	0.22 ± 0.13
16:1ω7t	1.62 ± 0.16	2.34 ± 0.08	2.58 ± 0.20	3.24 ± 0.24	2.76 ± 0.52	2.51 ± 0.60
c17:1	0.20 ± 0.03	0.27 ± 0.01	0.32 ± 0.02	0.38 ± 0.02	0.30 ± 0.03	0.29 ± 0.06
18:1ω9	6.55 ± 0.33	6.80 ± 0.20	5.88 ± 0.36	8.42 ± 1.06	7.29 ± 0.71	6.99 ± 1.03
18:2ω6	4.24 ± 0.56	4.45 ± 0.40	3.83 ± 0.41	7.25 ± 0.40	4.50 ± 0.81	4.85 ± 1.34
18:2b	0.14 ± 0.05	0.10 ± 0.01	0.12 ± 0.07	0.32 ± 0.14	0.22 ± 0.05	0.18 ± 0.11
a17:0	1.63 ± 0.10	0.96 ± 0.03	1.10 ± 0.04	1.45 ± 0.21	2.03 ± 0.16	1.43 ± 0.41
17:1ω8	0.56 ± 0.32	0.52 ± 0.02	0.40 ± 0.21	0.69 ± 0.35	1.07 ± 0.56	0.65 ± 0.38
18:2a	0.22 ± 0.06	0.17 ± 0.05	0.31 ± 0.10	0.73 ± 0.07	1.06 ± 0.38	0.50 ± 0.39
19:1b	0.87 ± 0.27	0.86 ± 0.12	1.29 ± 0.35	1.33 ± 0.61	1.94 ± 0.78	1.26 ± 0.59
20:5	0.60 ± 0.38	0.52 ± 0.24	0.56 ± 0.34	0.76 ± 0.48	0.98 ± 0.60	0.69 ± 0.42
20:0	0.80 ± 0.17	0.71 ± 0.24	0.77 ± 0.33	1.19 ± 0.49	2.41 ± 1.39	1.18 ± 0.90
Stable or variable						
15:0	0.87 ± 0.06	0.94 ± 0.04	0.95 ± 0.06	1.04 ± 0.07	0.97 ± 0.14	0.95 ± 0.09
16:1ω9	0.78 ± 0.08	0.64 ± 0.07	0.81 ± 0.09	0.69 ± 0.15	0.72 ± 0.15	0.73 ± 0.12
16:1ω7c	8.22 ± 1.69	7.41 ± 0.62	7.24 ± 0.32	8.23 ± 0.99	8.16 ± 1.21	7.85 ± 1.06
16:0	13.69 ± 2.85	14.04 ± 0.36	12.79 ± 1.23	14.40 ± 0.92	12.75 ± 1.55	13.54 ± 1.59
18:2c	0.19 ± 0.04	0.15 ± 0.03	0.14 ± 0.04	0.11 ± 0.05	0.18 ± 0.07	0.15 ± 0.05
20:4	0.47 ± 0.26	0.22 ± 0.09	0.15 ± 0.04	0.34 ± 0.17	0.36 ± 0.20	0.31 ± 0.19

2002). BASAL and SIR were significantly correlated with the gross N mineralisation rates ($r_S \sim 0.49$, $p = 0.002$, $n = 37$).

4. Discussion

Along the primary successional transect studied (alder/rowan, birch, birch/spruce, spruce I, spruce II) we hypothesized a concavely curvilinear response of basal respiration (BASAL) and microbial biomass (SIR) to changing organic matter quality, which was primarily revealed by an increasing C/N ratio and decreasing pH and net N mineralisation (Merilä et al., 2002).

In contrast to our hypothesis, BASAL and SIR were relatively stable along the transect. The result suggests that, at least within the rather short-term respiration-measuring period applied in this study, the decomposition of aged soil

organic matter occurs over a wide range of substrates at a relatively stable rate. Similarly, BASAL and SIR remained unchanged along a fertility gradient studied by Pennanen et al. (1999). However, Nohrstedt (1985), who examined microbial activity in forest floors using bulked samples of three samplings of one growing season, found a curvilinear response between respiration and the C/N ratio in the organic layer and concluded that optimum conditions for decomposition were within the C/N ratio range 20–30. In our study the samples taken during the most favorable temperature and moisture conditions in the field (BASAL in Jul-97 and BASAL and SIR in Jul-98) tended to show this pattern, i.e. BASAL and SIR increased slightly along the transect from alder/rowan to spruce I, but were again lower in spruce II, and thus partly supported our hypothesis. Microbial biomass N, measured in Jul-98 (Merilä et al., 2002) showed a similar response to the increase in the C/N ratio along the transect.

BASAL and SIR in spruce I site were occasionally surprisingly high. The highest rates were actually observed at stony and therefore dry and infertile spots with a thin, poorly decomposed organic layer. These patches obviously had a high density of fine roots and associated ectomycorrhizal hyphae. We may assume that mycorrhizal hyphae, still present in the sample after sieving, continued to respire after excision from their host, together with root exudates remaining in the sample, also provided a source of substrates for decomposing microbes, resulting in the relatively higher BASAL, SIR and FungPLFA measured in spruce I site. Moreover, the high microbial activity and biomass measured in this site may have reflected the favorable temperature and moisture conditions in the field, since poorly decomposed organic material evidently consists of labile C sources for decomposers. Poorly decomposed organic material may also partly explain the highest FungPLFA concentration in spruce I, because fungal communities have been found to play a dominant role in litter breakdown in the early stages of decomposition (Dilly et al., 2001).

SIR showed uniform seasonal variation in the birch, birch/spruce and spruce I sites. This seasonality may be due to variation in the temperature and moisture conditions in the field during the growing season (Merilä et al., 2002). The increase in soil temperature may also explain the increase in SIR rates from Jun-97 to Jul-97. At the time of sampling in Aug-97, the soil moisture content was low. Low field moisture, as a major factor controlling the activity of microbes (Schlentner and Van Cleve, 1985; Wagener and Schimel, 1998), may have caused the low SIR values as well as the low net N mineralisation rates in laboratory incubations (Merilä et al., 2002). BASAL was, however, very stable along the transect at that time. In Sep-97, again SIR increased, perhaps due, e.g. to the higher moisture content or the increase in labile C sources provided by the autumn litter fall. This result emphasizes that, although the actual measurements were carried out in constant moisture and temperature conditions in the laboratory, the conditions in the field (Buchmann, 2000) at the time of sampling appear to be the main reason for the observed variation.

qCO_2 showed no significant differences between the successional sites in three of the five samplings, and the birch/spruce (Jul-97) and birch and birch/spruce (Aug-97) in the other two samplings tended to show higher qCO_2 than the other sites. In a recent paper of Vance and Chapin (2001) the authors suggested that differences in qCO_2 between forest ecosystems may reflect several kind of disparities, such as differences in the proportion of inactive microbial biomass, in the degree of substrate limitation of microbial activity or in the metabolic rates, turnover, and growth efficiency of different microbial functional groups. In our study these factors seemed to counteract each other and few if any differences were apparent between the sites. While qCO_2 undoubtedly indicates microbial efficiency, this quotient appears to be too unspecific to reflect ecosystem development (Dilly and Munch, 1998).

As shown by NMS ordination of the PLFA data, the microbial community structure showed relatively clear differences along the transect and was well related to the C/N ratio and pH of the soil. It was possible on the basis of the similarities in the variation pattern along this transect, to divide the PLFAs into six groups, even though the indicative value of most of the single PLFAs is not clear. The most distinctive group in NMS ordination was formed by the samples from the alder/rowan site. The amount of PLFA 16:1ω5, which has been reported to be present in higher amounts in soil containing arbuscular mycorrhizal fungi (Olsson et al., 1995) was at its maximum in this site. The result is consistent with the fact that the understorey vegetation in the alder/rowan site was dominated by grasses and herbs (Merilä et al., 2002), which generally form this type of mycorrhizal association.

In the NMS ordination the samples taken in summer (Jun-97 and Jul-97) and in autumn (Aug-97 and Sep-97) tended to be separated from each other within each forest site. This was due to the fact that certain PLFAs (18:1, 19:1b, 20:0, 20:5) showed higher relative abundances in autumn than in summer. Although the indicative value of these PLFAs remains unclear, these changes in the PLFA pattern indicate seasonal changes in the microbial community structure.

In contrast to our hypothesis, substrate quality, as determined by the C/N ratio, did not generally account for the observed variation in microbial biomass and respiration. These variables remained relatively stable among the successional forest sites, in spite of the clear differences in the structure of the microbial community. As the data supported our hypothesis only during the most favorable weather conditions, we further hypothesize that the temperature and moisture conditions in the field at the time of sampling may account for the variation observed. The significance of abundant mycorrhizal hyphae,

especially in the late successional spruce sites, cannot be ruled out either.

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