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Inhibition of growth, and effects on nutrient uptake of arctic graminoids by leaf extracts – allelopathy or resource competition between plants and microbes?

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Abstract Previous research has shown that plant extracts, e.g. from boreal dwarf shrubs and trees, can cause reduced growth of neighbouring plants: an effect known as allelopathy. To examine whether arctic and subarctic plants could also be affected by leaching of phytochemicals, we added extracts from the commonly occurring arctic dwarf shrubs *Cassiope tetragona* and *Empetrum hermaphroditum*, and from mountain birch, *Betula pubescens* ssp. *tortuosa* to three graminoid species, *Carex bigelowii*, *Festuca vivipara* and *Luzula arcuata*, grown in previously sterilized or non-sterilized arctic soils. The graminoids in non-sterilized soil grew more slowly than those in sterilized soil. Excised roots of the plants in non-sterilized soil had higher uptake rate of labelled P than those in sterilized soil, demonstrating larger nutrient deficiency. The difference in growth rate was probably caused by higher nutrient availability for plants in soils in which the microbial biomass was killed after soil sterilization. The dwarf shrub extracts contained low amounts of inorganic N and P and medium high amounts of carbohydrates. *Betula* extracts contained somewhat higher levels of N and much higher levels of P and carbohydrates. Addition of leaf extracts to the strongly nutrient limited graminoids in non-sterilized soil tended to reduce growth, whereas in the less nutrient limited sterilized soil it caused strong growth decline. Furthermore, the N and P uptake by excised roots of plants grown in both types of soil was high if extracts from the dwarf shrubs (with low P and N concentrations) had been added, whereas the P uptake declined but the N uptake increased after addition of the P-rich *Betula* extract. In contrast to the adverse extract effects on plants, soil microbial respiration and soil fungal biomass (ergosterol) was generally stimulated, most strongly after addition of the *Betula* extract. Although we cannot exclude the possibility that the reduced plant growth and the concomitant stimulation of microbial activity were caused by phytochemicals, we believe that this was more likely due to labile carbon in the extracts which stimulated microbial biomass and activity. As a result microbial uptake increased, thereby depleting the plant available pool of N and P, or, for the P-rich *Betula* extract, depleting soil inorganic N alone, to the extent of reducing plant growth. This chain of events is supported by the negative correlation between plant growth and sugar content in the three added extracts, and the positive correlation between microbial activity, fungal biomass production and sugar content, and are known reactions when labile carbon is added to nutrient deficient soils.

Key words Allelopathy · *Betula pubescens* ssp. *tortuosa* · *Cassiope tetragona* · *Empetrum hermaphroditum* · Plant-microbe competition

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Introduction

Vast areas of heaths and fellfields in the Arctic are dominated by the ericoid dwarf shrubs *Cassiope tetragona* and *Empetrum hermaphroditum* (Bliss and Matveyeva 1992). The reasons for the dominance of single or few plant species in natural systems are complex, and interpretation of data on interactions between plant species in the field is difficult. As an example, both negative and positive interactions have been found between the arctic sedge *Carex bigelowii* and the two dwarf shrub species above (Carlsson and Callaghan 1991). Such effects could be due to shelter (Carlsson and Callaghan 1991), competition for light and nutrients, allelopathic release of compounds from leaves, stems or roots, etc.

Recent studies have shown that *E. hermaphroditum* from boreal forest strongly reduces the seed germination, growth and nutrient uptake by roots and mycorrhizae of *Pinus silvestris* by the release of allelopathic agents from the leaves (Hytönen 1992; Zackrisson and Nilsson 1992; Nilsson et al. 1993; Nilsson 1994), from which an active extract component (5-methoxy-3,3′-dihydroxy, dihydrostilbene) has been identified (Odén et al. 1992). It would therefore not be surprising if *E. hermaphroditum* and other heath and non-heath species had the same effects on neighbouring plants in arctic ecosystems. For instance, the leaves of *Cassiope tetragona* contain flavonoids (Denford and Karas 1975) and phenolic compounds (Harborne and Williams 1973) which may have a similar effect as those found in other species claimed to have an allelopathic effect, e.g. *Eucalyptus* spp. (Lisanework and Michelsen 1993). Thus, experimental studies of possible allelopathic effects on plant growth and nutrient acquisition could facilitate the understanding of competition between plants in arctic environments, but to the best of our knowledge no such research has been undertaken previously.

The aim of this investigation was to study the effect of addition of leaf extracts of *Cassiope tetragona*, *Empetrum hermaphroditum* and *Betula pubescens* ssp. *tortuosa* on soil microbial activity, soil fungal biomass, biomass production and nutrient uptake of three arctic graminoids, *Carex bigelowii*, *Festuca vivipara* and *Luzula arcuata* grown in pots with arctic soils.

Leaf extracts of *B. pubescens* ssp. *tortuosa* were included because long-term global warming as predicted by General Circulation Models may result in this species increasing its production in the southern part of its area of distribution (Kellomäki and Kolström 1994) and expanding northwards and to higher altitudes (Kullman 1993), and because its leaves are rich in secondary metabolites (Bryant and Reichardt 1992) which could be potentially phytotoxic (Hytönen 1992). The graminoids were chosen as target plants because they are widespread and common in a range of Arctic vegetation types (Fredriksen 1981; Carlsson and Callaghan 1991; Bliss and Matveyeva 1992) and represent different strategies of growth: *Carex bigelowii* stores large amounts of nutrients in rhizome and roots which are used as reserves when nutrient uptake is constrained (Jónsdóttir and Callaghan 1990; Carlsson and Callaghan 1991), whereas *F. vivipara*, amongst other tundra grasses (Chapin and Shaver 1989; Berendse and Jonasson 1992), responds quickly to pulses of nutrients released in nutrient limited systems.

Separation of allelopathic effects from those of nutrient limitations can be difficult (Michelsen et al. 1993; Nilsson 1994) as plant-plant or plant-microbe resource competition may affect experiments on allelopathy. Furthermore, phytotoxins may inhibit microbes, e.g. mycorrhizal fungi (Nilsson et al. 1993). We added extracts to sterilized and non-sterilized soil, followed by measurements of fungal biomass and microbial respiration at the end of the experiment, i.e. for micro-organisms in sterilized soil after their recolonization. Micro-organisms may strongly affect plant growth in non-arctic (Harte and Kinzig 1993; Jonasson et al. 1995) and arctic soils; recent research has shown strong nutrient immobilization by the microbial community in nutrient limited ecosystems and significant competition with higher plants for nutrients (S. Jonasson et al., to be published). For this reason we examined whether reduced plant growth and nutrient uptake ascribed to phytotoxins could be due to enhanced microbial nutrient demand caused by addition of carbohydrates with the plant extracts to the soil.

As we anticipated that exposure to leaf extracts, either through allelopathy or microbial competition for nutrients would impose nutrient limitations on the graminoids, we undertook root uptake bioassays with ^{15}N and ^{32}P enrichment (Harrison and Helliwell 1979; Harrison et al. 1991; Jones et al. 1991; Dighton et al. 1993). Since excised roots from plants grown under nutrient deficient conditions take up greater amounts of the nutrient element (identified by its tracer) from solution than roots from plants grown in optimum conditions, these bioassays provide an index of nutrient limitations for plant growth.

Material and methods

Collection of leaf material and preparation of leaf extracts

Branches of a few large specimens of *Cassiope tetragona* and *Empetrum hermaphroditum* were collected on 11 September 1993, from a fellfield on mount Slåttatjåkka near Abisko in Northern Swedish Lapland, 1150 m a.s.l. The climate is montane subarctic, with a growing season of approximately 3 months, lasting from mid- to late June to early-mid-September. See Havström et al. (1993) and Jonasson et al. (1993) for a more detailed account of the climate, vegetation and soil parameters at the site. The branches were immediately flown to Copenhagen and stored at 4°C for 1 week. After leafless stem sections had been removed, the branches were cut into pieces of 2–4 cm lengths, thoroughly mixed and kept at –20°C until use.Leaves of *Betula pubescens* ssp. *tortuosa* were collected close to the tree line, at 450 m altitude, near Abisko Scientific Research Station. The senescent leaves were shaken off branches of a few trees at the time of leaf fall in late September. The leaves were air-dried for 48 h in order to reduce moisture on the leaf surfaces, mixed and frozen.

Leaf extracts were obtained by shaking fresh leaves or shoots corresponding to a dry weight of 25 g for 48 h at 4°C in 1 l distilled water. This concentration was chosen because it corresponds to that in snowmelt water under dense *E. hermaphroditum* vegetation, and has proved toxic to seed germination (Zackrisson and Nilsson 1992; Nilsson et al. 1993). The extracts, which were freshly produced before each weekly addition, were filtered through standard filter paper with a pore size of 11 μm. Distilled water was used as control. The procedure was equivalent to that of Zackrisson and Nilsson (1992) and Nilsson et al. (1993) for *E. hermaphroditum* although we did not dry our material before freezing. This was because we considered that in situ freezing of plant material was more likely than drying at the collection sites during autumn.

Collection of assay plants and soil

Specimens of *Carex bigelowii*, *Festuca vivipara* and *Luzula arcuata* were collected in intact humus mats of approximately

20 × 20 cm and 5–8 cm depth within a few square metres at Slåttatjåkka on 11 September 1993. The plants were immediately flown to Copenhagen and placed in trays in a greenhouse with a day and night temperature of 10°C and 5°C, respectively, and with a daylength of 16 h.

Soil used subsequently as growth medium was collected as 10-cm-deep plugs of humus with an auger from a tree-line heath (450 m a.s.l.) close to Paddustieva (near Abisko) on 3 September, and kept refrigerated at 4°C. For details of the climate, vegetation and soil of the site, see Havström et al. (1993) and Jonasson et al. (1993).

Preparation of soil and plant material

Pieces of mosses and coarse and fine roots were removed from the soil within 4 days after collection, after which the soil was frozen. The soil was thawed and mixed on 28 September in Copenhagen. Half of the soil was autoclaved twice for 1 h at 120 C. One volume of soil (20 ml) was mixed with 5 volumes of prewashed expanded clay (Leca) with a particle size of 2–4 mm and transferred to pots with a volume of 125 ml.

Tillers of an equal size were carefully extracted from the humus mats for use as assay plants. Dead leaves were removed and the shoots were washed clean of soil adhering to the roots. Young tillers of *C. bigelowii* with three leaves were cut into modules consisting of the shoot together with a 2 cm piece of rhizome and attached roots cut at 1.5 cm distance from their base. This was done in order to increase the uniformity of the plants. Shoots of *L. arcuata* and tussock-forming *F. vivipara* were extracted and trimmed in the same way. Leaves exceeding five cm in length (*F. vivipara*) were cut at five cm distance from the base and the roots were pruned to 1.5 cm length.

Treatments and experimental design

On 30 September half of the shoots were planted in the pots with the mixture of soil and expanded clay which had been sterilized and the other half in the non-sterilized soil-Leca mixture.

The pots with *F. vivipara* were placed in an automatically controlled growth chamber with cooling from below the pots. The temperature was adjusted to mean diurnal air temperature fluctuations at the high (1150 m) altitude site at Slåttatjåkka (Havström et al. 1993), within the limits of 4.5°C (night) and 12.2°C (day). The temperature below the pots was approximately 2°C lower than the air temperature at daytime whereas at night-time the temperature was the same above and below the pots. The irradiance from 7 a.m. to 11 p.m. was 280 $\mu E\ m^{-2}\ s^{-1}$ at leaf level, yielding a daylength of 16 h.

Due to limited space in the growth chamber, the pots with *C. bigelowii* and *L. arcuata* were placed in a greenhouse with temperature control facilities, with a day and night temperature of approximately 13°C and 5°C, respectively, and with a daylength of 16 h. At midday on 7 October in full sun the light intensity was 540 $\mu E\ m^{-2}\ s^{-1}$ at leaf level, whereas it was 210 $\mu E\ m^{-2}\ s^{-1}$ when overcast.

All pots were watered twice weekly with 12.5 ml of a dilute nutrient solution (Table 1). It could be argued that the use of NO_3-N fertilizer is inappropriate as NO_3-N is infrequent in the arctic soils (Nadelhoffer et al. 1992; Jonasson et al. 1993). However, many arctic plants have the potential to utilize NO_3-N (Atkin and Cummins 1994), and the graminoids used in our assay showed nitrate reductase activity at the field sites where the plant material was collected (Jónsdóttir and Callaghan 1990; Michelsen et al. 1995.

From day 17 and until harvest, the plants were watered once weekly with an additional amount of 10 ml extract of either *Betula pubescens*, *Cassiope tetragona*, *Empetrum hermaphroditum* (extracts will be identified by the generic names of the species), or with distilled water. Hence, for each of the three assay species, the experiment was a 2 × 4 factorial, with the following factors: soil type (sterilized versus non-sterilized soil) and extract exposure (*Betula*, *Cassiope*, *Empetrum*, and distilled water). For *Carex bigelowii* and *F. vivipara* there were 11 replicates in each treatment, whereas there were 9 replicates for *L. arcuata*.

The pots of each assay species were completely randomized at the onset of the experiment, and twice during the experiment. Roots growing through the bottom of the pots towards the end of the experiment were pruned at weekly intervals.

Analysis of plant growth and biomass production

The number of leaves and the total green leaf length of the plants (including the pigmented red leaves of *F. vivipara*) were estimated at approximately 14 days intervals, leaf lengths being measured from the base of the leaf blade.

At day 90 we harvested *L. arcuata*, whereas *Carex bigelowii* and *F. vivipara* were transferred from Copenhagen to Merlewood Research Station (on day 95) and harvested there later. *L. arcuata* shoots and roots were separated, dried at 60°C and weighed, and samples of the soil was taken for analysis of P and N (see below). *C. bigelowii* and *F. vivipara* were placed in a growth chamber at Merlewood and kept under similar light, temperature and watering regimes as previously until they were harvested on day 117 and day 104, respectively. The harvested *C. bigelowii* and *F. vivipara* were analysed for N content, N and P uptake in excised roots, and for mycorrhizal colonization. The soil was used for measurements of respiration and estimation of fungal biomass. The different analyses performed on *L. arcuata* and the two other species resulted from practical difficulties in transporting all plant material to Merlewood and processing the very large amounts of samples there in a relatively short period of time. Partitioning of the material between the two laboratories also made it possible to measure a wider variety of responses than would have been possible by using only one laboratory.

Analysis of nutrients and polysaccharides in extracts, and of nutrients in soil and plants

Ten ml of leaf extracts were digested in 5 ml of concentrated H_2SO_4 with added Se and analysed for P with the molybdenum-blue method, and for NH_4-N with the salicylate method, using a Hitachi U-2000 spectrophotometer in both cases. NO_3-N was determined by the cadmium reduction method by flow injection analysis with an Aquatec System (Tecator, Höganäs, Sweden). The leaf extract content of labile polysaccharides was measured by the phenol-sulphuric acid method (Dubois et al. 1956) at 490 nm.

Extractable P, NH_4-N and NO_3-N in *L. arcuata* soil was determined with the same methods as above in subsamples of 10 g fresh soil and Leca from the pots extracted in 50 ml of 0.5 M K_2SO_4 for 1 h. The extracts were filtered through Munktell OOK filter paper, and frozen until analysis. The remaining soil was dried at 55°C to constant weight, and the water content was calculated. Soil concentrations of the extractable ions were calculated from the concentrations in the extract corrected by soil water content.

Total N in soil and leaf extracts was analysed as above after digestion of 0.1 g material in 5 ml of concentrated H_2SO_4 with added Se.

Analysis of P and N uptake by excised roots and mycorrhizal colonization

After the harvest of *Carex bigelowii* and *F. vivipara* the roots were carefully removed from the soil, the remaining soil was washed off, and the roots were blotted dry. The shoots were separated from the roots (shoots of *C. bigelowii* were further separated into leaves and rhizome), dried at 60°C and weighed. The fresh, weighed roots were divided into two subsamples for use in the

bioassay of P (Harrison and Helliwell 1979) and N uptake (Jones et al. 1991).

The roots for the P-deficiency bioassay were placed for 30 min in 5×10^{-4} M $CaSO_4$ solution to maintain cell membrane integrity and to leach out physically sorbed P in the root free-space. They were then placed for 15 min in a solution of 5×10^{-4} M $CaSO_4$, 5×10^{-6} M KH_2PO_4 and 1.33 MBq ^{32}P (as orthophosphate) l^{-1}. The samples were subsequently washed for 5 min and counted in glass vials containing 15 ml water for Cerenkov radiation in a Canberra Packard 2000CA liquid scintillation spectrometer. The roots were removed from the vials, blotted and weighed, and the vials were recounted to correct for any isotope that had not been metabolically absorbed. The activity was corrected for colour quench, background and isotope decay. The root uptake of ^{32}P label was calculated as pg P mg^{-1} fresh weight of root 15 min^{-1}.

The roots used in the P bioassay were kept in FAA and later, after ^{32}P decay, analysed for mycorrhizal colonization by the grid-length method, after clearing in KOH (1 h, 80°C) and staining with Trypan Blue (10 min, 80°C) as in Michelsen et al. (1993).

The N-deficiency bioassay followed the procedure of Jones et al. (1991). The roots were pretreated in 5×10^{-4} M $CaCl_2$ for 30 min. and transferred to 5×10^{-4} M $CaCl_2$ containing 1 mg l^{-1} N as NH_4Cl, labelled with ^{15}N (20% enrichment). They were left in the solution for 2 h, washed for 15 min, dried, weighed and finely cut. Samples of about 12 mg root material were put into pre-weighed tin capsules, redried at 105°C, cooled, crimped, re-weighed and analysed for ^{15}N. The analyses were carried out on a Roboprep, a Dumas-type continous-flow CHN analyser, coupled to a Tracermass isotope ratio mass spectrometer (Europa Scientific Ltd, Crewe, UK). Atom percentage excess ^{15}N was converted to uptake rate of N as ng mg^{-1} root 2 h^{-1}. The total N in the roots was also calculated, after subtraction of N which had been taken up during the bioassay.

Soil respiration and fungal biomass estimation

The respiration rate of the soil from the pots with *Carex bigelowii* and *F. vivipara* was measured 1 week after the harvest of the plants. Twenty grams fresh weight of soil with Leca (equivalent to about 10 g of dry soil and Leca), wetted to field capacity were placed in gas-tight 500-ml jars. The jars were flushed with CO_2-free air, closed with a rubber stopper (Suba-Seal, UK) and incubated at 10°C for 24 h. The CO_2 evolved from the system was determined by infra-red gas analysis (Durson et al. 1993) with a Series 225 IRGA (The Analytical Co. Ltd, Hoddesdon, UK). Soil samples of 10 g were dried to constant weight in order to obtain soil fresh weight to dry weight conversion factors, and the CO_2 released was expressed in relation to the soil dry weight.

The live fungal biomass in the soil from pots with *C. bigelowii* and *F. vivipara* was estimated using the ergosterol method (Grant and West 1986; Antibus and Sinsabaugh 1993; Hill et al. 1993). One week after the harvest, 5 g of soil (fresh weight) was placed in 20 ml of HPLC-grade methanol and kept in the dark at 2°C until analysis. A further 10 ml methanol was added, and the samples were refluxed for 2 h, filtered through Whatman GF/A filters and saponified with 5 ml of 4% KOH for half an hour. After three-step pentane extractions of the samples in separation funnels using 10, 5 and 5 ml pentane, and evaporation of the pentane to dryness, the samples were redissolved in 1 ml methanol, injected into a reverse-phase HPLC (LDC/Milton Roy Spectro Monitor 3000) and measured at 282 nm.

For sterilized soil, microbial respiration and ergosterol content yield a measure of recolonisation of microorganisms 3.5–4 months after sterilization.

Statistical analysis

Leaf number and leaf length were analysed using repeated measurements analysis of variance. All other parameters were analysed using two-way ANOVA with extract type and soil type as main effects. The means of the eight treatments were compared with Tukey's test. Prior to analysis the data on soil extractable nutrients were log(n+1) transformed. Correlations are described using Pearson correlation coefficients. All statistical analyses were performed with SAS (SAS Institute 1988).

Results

Nutrients in leaf and soil extracts

The sterilized soil had a higher content of K_2SO_4 extractable P, NH_4 and NO_3 than the non-sterilized soil (Table 1), probably mainly a result of release of nutrients from the microbial pool after the sterilization.

The P content of the *Betula* leaf extract was more than two orders of magnitude higher than that of the other extracts, and more than three orders of magnitude higher than the concentration in the nutrient solution and in solution of the non-sterilized soil. The NH_4 content of the *Betula* extract also exceeded that of the other extracts but was lower than that of the soils. The NO_3 content of the plant extracts and soils used in the experiment was negligible (Table 1).

Leaf extract addition significantly affected the P and NO_3 extractable from soil at the end of the experiment (ANOVA $P < 0.0001$). Due to the high P concentration in the *Betula* extract, the extractable soil P was also high

Table 1 Inorganic chemical characteristics of leaf extracts and soil, and labile polysaccharides in leaf extracts[a] (*n.d.* not determined)

	pH in H_2O	EC (µS/cm)	P (µg/g)	NH_4-N (µg/g)	NO_3-N (µg/g)	Total N (µg/g)	Labile polysaccharides (µg/g)
Distilled water	6.12	7	0.0	0.00	0.00	0.00	0
Cassiope tetragona extract	4.60	137	0.3	0.02	0.00	3.25	582
Empetrum hermaphroditum extract	4.55	135	0.4	0.00	0.00	0.00	838
Betula tortuosa extract	5.63	631	46.0	0.65	0.00	1.87	1129
Nutrient solution used	4.69	14	0.045	0.00	4.91	n.d.	n.d.
Sterilized soil[b]	n.d.	175	7.7	29.8	0.39	7000	n.d.
Non-sterilized soil[b]	7.12	139	0.0	2.4	0.00	8100	n.d.

[a] The data are means of measurements of 2–4 different batches of extracts and soil. Variation from the mean was less than 5% and was due more to instrumental precision than to variability between batches

[b] The soil chemical characteristics were measured before addition of expanded clay. Soil P, NH_4 and NO_3 are K_2SO_4 extractable fractions

Table 2 Extractable soil P, NH_4 and NO_3 after harvest of *Luzula arcuata* grown in soils treated with leaf extracts of *Cassiope tetragona*, *Empetrum hermaphroditum* or *Betula tortuosa*, or distilled water (control); $n = 9$, mean ± standard error. The means of the eight treatments are compared with Tukey's test at P = 0.05; for each variable, means with same letter are not significantly different

	P (μg/g)	NH_4-N (μg/g)	NO_3-N (μg/g)
Sterilized soil			
Control	5.2 ± 0.5 b	0.7 ± 0.1 b	88.4 ± 15.7 a
Cassiope extract	3.7 ± 0.4 b	1.2 ± 0.3 ab	40.9 ± 4.9 bc
Empetrum extract	4.5 ± 0.7 b	1.9 ± 0.4 a	29.0 ± 4.3 c
Betula extract	69.5 ± 4.5 a	0.9 ± 0.1 ab	8.6 ± 1.3 d
Soil not sterilized			
Control	0.7 ± 0.1 d	1.7 ± 0.4 ab	79.3 ± 6.4 a
Cassiope extract	0.7 ± 0.2 d	1.2 ± 0.3 ab	50.5 ± 5.8 abc
Empetrum extract	1.5 ± 0.2 c	0.7 ± 0.1 b	30.8 ± 2.8 c
Betula extract	84.3 ± 2.5 a	0.7 ± 0.2 b	3.8 ± 1.1 e
ANOVA ($F;^{P}$)			
Soil type	$2.14^{0.1480}$	$0.18^{0.6764}$	$0.33^{0.5702}$
Extract	$821.93^{0.0001}$	$1.46^{0.2335}$	$37.38^{0.0001}$
Soil*Extract	$12.56^{0.0001}$	$5.95^{0.0012}$	$0.56^{0.6409}$

in the treatments with the *Betula* extracts at the end of the experiment (Table 2). Addition of *Cassiope* and *Empetrum* extracts had only marginal influence on the final soil P and NH_4 concentrations. In contrast, the NO_3 concentration was low in the *Betula* treatments, high in the controls and intermediate in the soils exposed to *Cassiope* and *Empetrum* extracts.

The extracts also contained appreciable amounts of polysaccharides. *Betula* extracts contained almost twice as much as the *Cassiope* extract, whereas the *Empetrum* extract was intermediate (Table 1). There was a striking negative correlation between the amount of polysaccharides added with the extract and the final soil NO_3 (Tables 1, 2).

Plant growth and biomass production

Plants grown in the sterilized soil formed more and longer leaves (Fig. 1; $P < 0.0001$ for all species), and shoot and root dry weights were greater than in plants grown in non-sterilized soil (Fig. 2; $P < 0.001$) exposed to the same treatments, irrespective of the species tested. The number of green leaves and the total green leaf length of *F. vivipara* changed little during the course of the experiment with non-sterile soil, whereas it increased steadily in sterilized soil, except in plants treated with *Betula* extract (Fig. 1). This resulted in more than a threefold increase of the biomass of *F. vivipara* controls of the sterilized soil as compared to the final biomass in the non-sterilized soil. The effects on *L. arcuata* leaf production were similar to those on *F. vivipara*, but leaf production was smaller and ceased about day 76, corresponding to the length of the growing season at the site where the plants were collected. *Carex bigelowii* continued to produce leaves in all treatments until day 76, although at a lower rate in non-sterile soil (Fig. 1). After day 76 the main shoots senesced and new tillers were produced.

The treatment with leaf extracts significantly affected leaf number and leaf length of *F. vivipara* and *Carex bigelowii* ($P < 0.0001$). There was a significant interaction between the effect of soil sterilization and extract exposure on leaf production and leaf and root biomass of *F. vivipara* ($P < 0.0001$) as the application of leaf extracts reduced the growth of *F. vivipara* in sterilized soil only (Figs. 1, 2). For *F. vivipara* in sterilized soil, the leaf production during the course of the experiment (Fig. 1), and the final leaf biomass (Fig. 2) was highest in the distilled water treatment, followed in descending order by the treatments with *Cassiope* extract, *Empetrum* extract, and *Betula* extracts. *L. arcuata* showed a similar response, but the difference between treatments was less. In *Carex bigelowii* all extract treatments showed a significant reduction in their leaf number and length compared to controls, and extracts tended to reduce the leaf biomass (but with no difference between the type of extract) whereas the rhizome and root biomass was not significantly affected.

Plant nutrient uptake

The uptake of P by excised roots was significantly higher for *Carex bigelowii* and *F. vivipara* grown in non-sterilized soil than in sterilized soil ($P < 0.001$ and $P < 0.05$, respectively; Fig. 3). The plants treated with *Cassiope* and *Empetrum* extracts had consistently (although not always significantly) higher uptake of P than controls, i.e. they were subjected to higher P deficiency as a result of the treatment with leaf extracts. In contrast, the P uptake of roots of plants treated with extract of *Betula* was lower than that of the controls; i.e. they were subjected to less P limitation, a result which is consistent with the high P concentration in the leaf extract of *Betula*.

In contrast, the root N uptake was highest in plants treated with *Betula* extracts, intermediate in roots exposed to *Cassiope* and *Empetrum* extracts and lowest (except for *F. vivipara* grown in sterilized soils) in controls (Fig. 3). This suggests that addition of leaf extracts caused N deficiency in the plants, which is confirmed by their low N concentration in leaves and roots as compared to the controls (Fig. 4).

Less than 1% of the root lengths of the graminoids were colonized by arbuscular mycorrhizal fungi in non-sterilized soil, and their abundance was unrelated to extract exposure (no results presented). This scarcity corre-

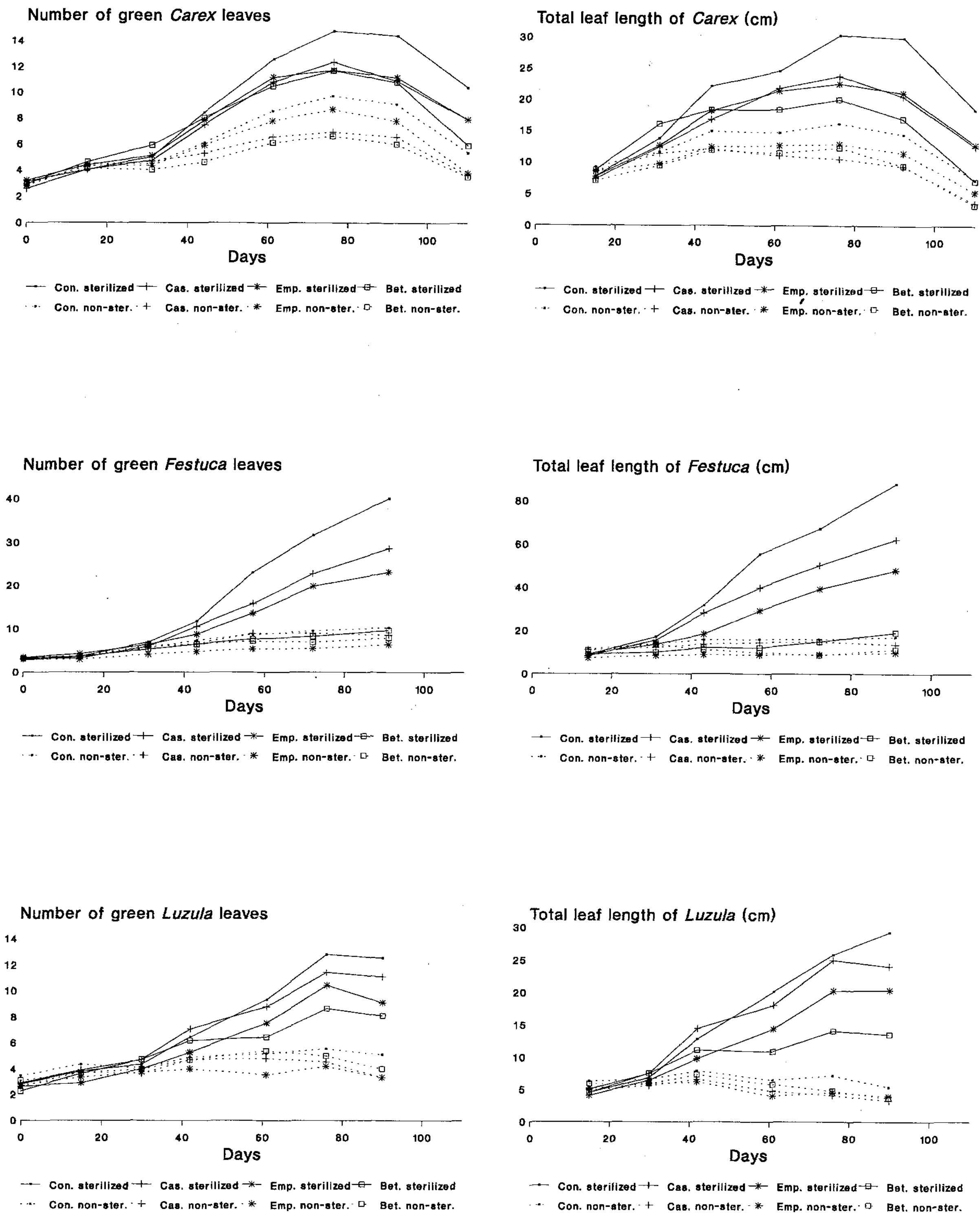

Fig. 1 Number of green leaves, and the total leaf length of *Carex bigelowii*, *Festuca vivipara* and *Luzula arcuata* exposed to distilled water or leaf extracts of *Cassiope tetragona* (*Cas*), *Empetrum hermaphroditum* (*Emp*) and *Betula tortuosa* (*Bet*), and grown in sterilized and non-sterilized soil (n = 11, except for *L. arcuata* where n = 9). The weekly addition of leaf extracts began at day 17

sponded to the absence of arbuscular mycorrhizal fungi in roots of graminoids in situ, at the site where the soil used in the experiment was collected (Michelsen et al. 1995).

Soil respiration and fungal biomass

The respiration was marginally higher in non-sterilized soil in which *Carex bigelowii* had grown than in the cor-

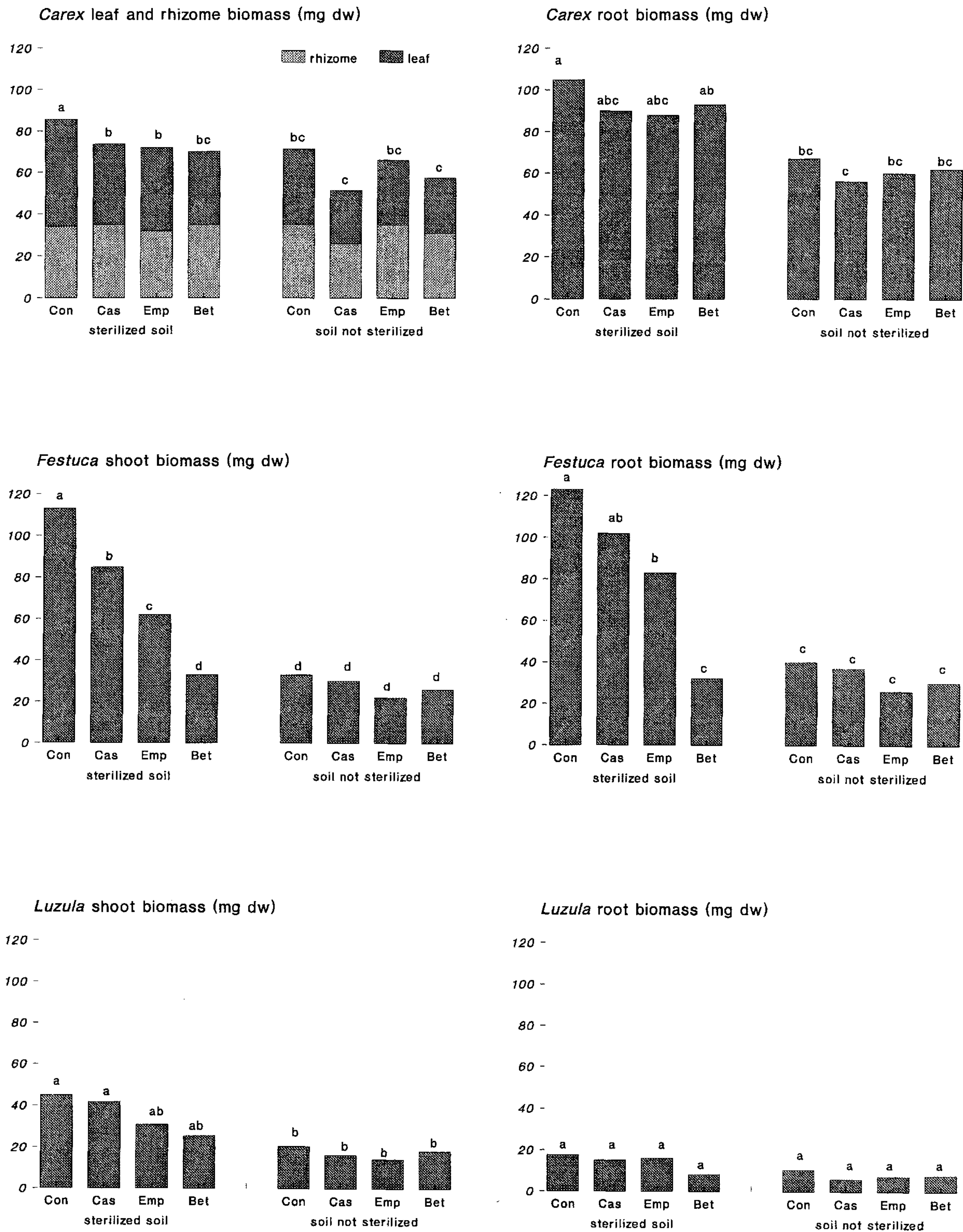

Fig. 2 Shoot and root biomass of *Carex bigelowii*, *Festuca vivipara* and *Luzula arcuata* exposed to distilled water or leaf extracts of *Cassiope tetragona* (*Cas*), *Empetrum hermaphroditum* (*Emp*) and *Betula tortuosa* (*Bet*). The means of the eight treatments are compared with Tukey's test at $P = 0.05$; means with same letter are not significantly different ($n = 11$, except for *L. arcuata* where $n = 9$). For *Carex bigelowii* shoots the test results of leaf biomass are presented; rhizome biomass showed no differences between treatments

responding sterilized soil ($P < 0.05$; Fig. 5), but similar in sterilized and non-sterilized soil of *F. vivipara*. However, the soil ergosterol content, a measure of the soil fungal biomass, was 6 (*C. bigelowii*) and 3–5 (*F. vivipara*) times higher in the non-sterilized soil (Fig. 6); $P < 0.0001$). This pattern indicates microbial recolonization during the months after sterilization. The proportionally high CO_2 flux from sterilized soil, in spite of the low fungal biomass, indicates that bacteria had recolo-

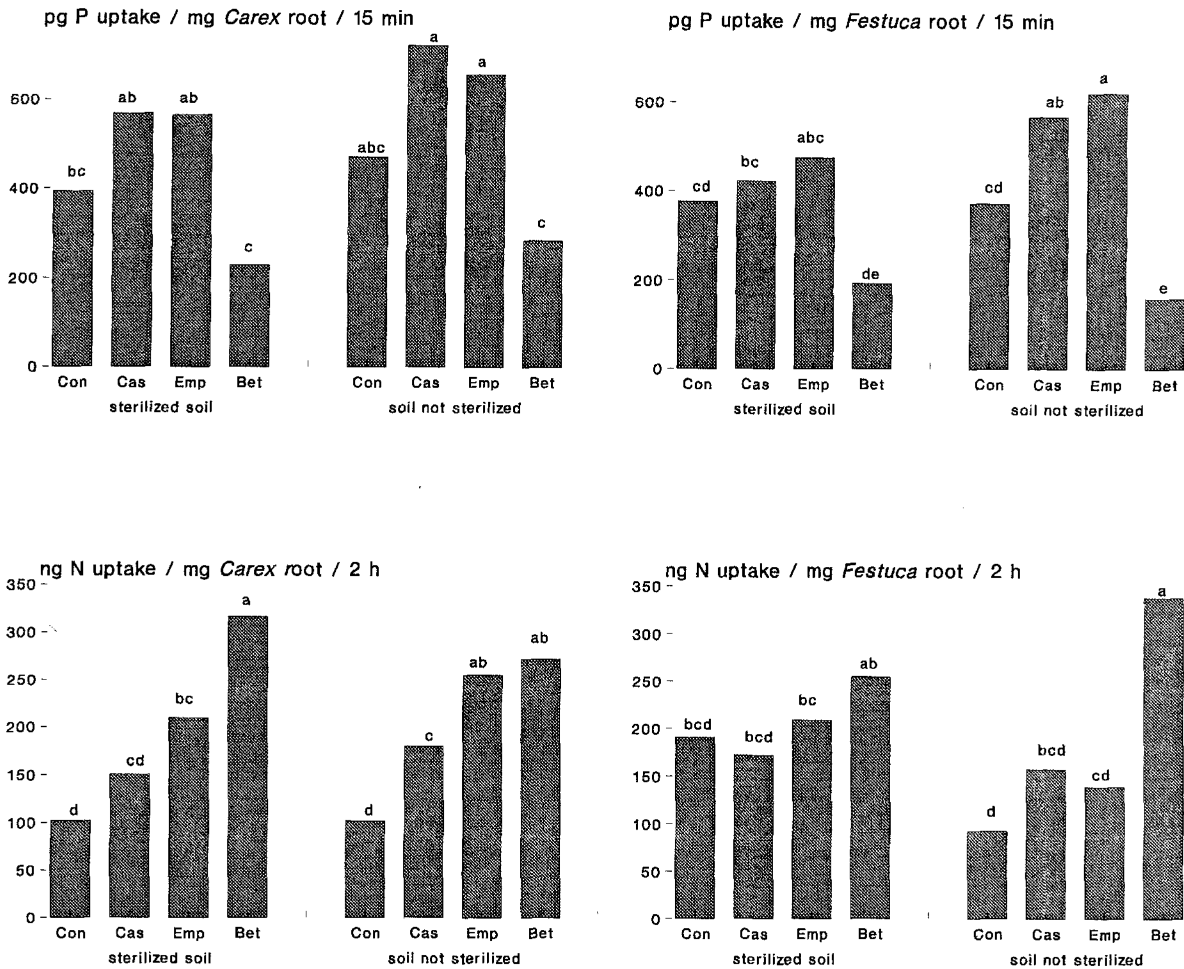

Fig. 3 N and P bioassay uptake of excised roots of *Carex bigelowii* and *Festuca vivipara* exposed to distilled water or leaf extracts of *Cassiope tetragona* (*Cas*), *Empetrum hermaphroditum* (*Emp*) and *Betula tortuosa* (*Bet*). The means of the eight treatments are compared with Tukey's test at $P = 0.05$; means with same letter are not significantly different ($n = 11$).

nized the sterilized soil more rapidly than the fungi, most likely from surrounding pots with non-sterilized soil.

The microbial respiration in sterilized growth medium of *Carex bigelowii* was highest in pots treated with *Betula* extracts, less with the *Empetrum* and *Cassiope* extract treatments and least in the controls. That is, the microbial activity tended to be high in pots where plant growth was low, and vice versa ($r = -0.246$, $P = 0.121$). In non-sterilized soil, where there was variation in plant biomass, the microbial respiration was similar to that of the non-sterilized soil: highest with *Betula* extracts, less with *Empetrum* and *Cassiope* extract and least in the controls. Respiration was positively correlated with the N uptake by excised roots of *Carex bigelowii* ($r = 0.676$, $P < 0.0001$) and negatively so with the shoot N concentration ($r = -0.416$, $P < 0.0001$), which shows that plants in soil with high microbial respiration were strongly N deficient.

In the non-sterilized growth medium of *F. vivipara* the respiration was highest after treatment with *Betula* extract (which caused the greatest reduction in growth), intermediate in the growth medium treated with *Empetrum* extract, and lowest with the *Cassiope* and control treatments (Fig. 5). Hence, the microbial respiration was negatively correlated with the plant biomass ($r = -0.370$, $P < 0.01$). In sterilized soil the respiration in the *Betula* treatment exceeded that of the two other extracts, but not that of the controls. The respiration of the sterilized *F. vivipara* control soil was not lower than soils from pots treated with extracts, but the coefficient of variation in the controls was high (17% as compared to a mean CV of 10% in pots treated with extracts), partly because one control soil had a respiration rate which was more than 50% higher than the mean in this treatment. It is likely that the soils of *F. vivipara* included a small amount of extremely fine (respiring) roots as these were difficult to remove intact from the humus soil. This would have the strongest effect on the respiration of soils from pots with the largest biomass, i.e. the controls in sterilized soil.

In the non-sterilized soil the ergosterol content was correlated with the soil respiration [$r = 0.359$, $P < 0.05$ (*Carex bigelowii*); $r = 0.439$, $P < 0.01$ (*F. vivipara*)]. For *Carex bigelowii* the ergosterol was higher in the *Betula* treatment than in the controls, a similar pattern to the soil respiration. No differences were found between extract treatments in the case of *F. vivipara*.

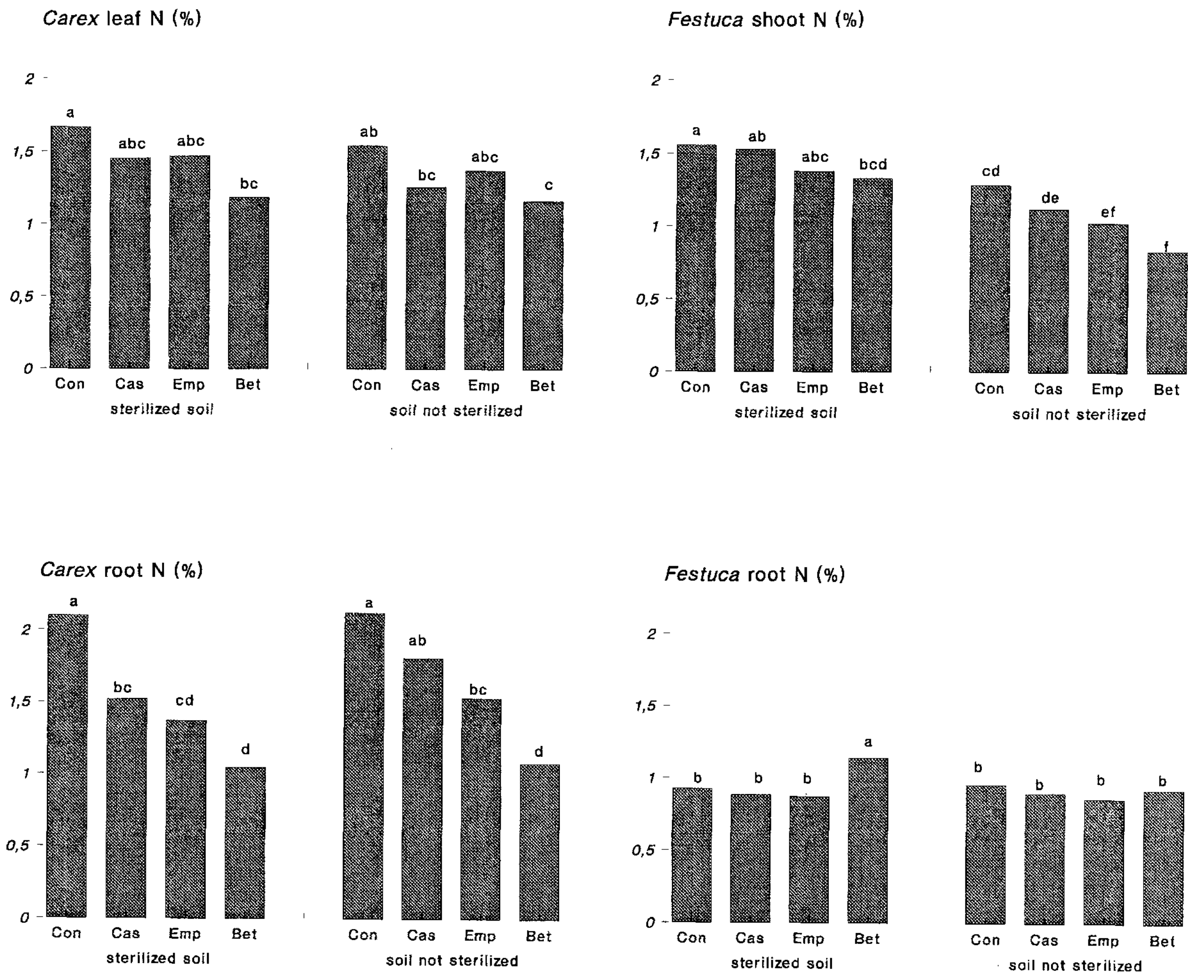

Fig. 4 N concentration in leaves and roots of *Carex bigelowii* and *Festuca vivipara* exposed to distilled water or leaf extracts of *Cassiope tetragona* (*Cas*), *Empetrum hermaphroditum* (*Emp*) and *Betula tortuosa* (*Bet*). The means of the eight treatments are compared with Tukey's test at $P = 0.05$; means with same letter are not significantly different ($n = 11$)

Discussion

The growth of the three graminoid species in sterilized soil was strongly reduced by addition of leaf extracts of *Cassiope*, *Empetrum* and *Betula* (Figs. 1, 2). Similar effects on plant biomass, e.g. of *Empetrum* extracts on neighbouring *Pinus sylvestris* (Nilsson et al. 1993; Nilsson 1994), have been ascribed to allelopathic compounds. However, based on the nutrient and carbohydrate concentration in soils and extracts and the plant and microbial performance, we believe that the effects on the plants did not necessarily result from allelochemicals but rather from labile carbon in the extracts, an energy source which stimulated microbial activity. This promoted microbial nutrient uptake which depleted the plant available-nutrient pool and increased the nutrient deficiency in plants to the extent of reducing plant growth.

Plant growth in the non-sterilized soil was probably principally limited by P rather than by N, as reported elsewhere for organic tundra soils (Nadelhoffer et al. 1992; S. Jonasson et al., to be published). This is indicated by the low initial P concentration (Table 1) and the small soil P increase during the course of the experiment, in spite of the addition of P in the nutrient solution. In contrast, NO_3 had increased strongly at the time of harvest (Table 2, controls). The strong P limitation led to slow plant growth (Fig. 1). Once P was made available to the plants in larger amounts through soil sterilization, the plants responded by strongly enhanced growth.

All *Carex bigelowii* and *L. arcuata* ceased to grow towards the end of the experiment, probably because these species are adapted to the short growing season in tundras. *F. vivipara* exhibited a different strategy. This species continued to grow during the last phase of the experiment, and generally with a higher growth rate in sterilized soil. This was not related to the inorganic soil N concentration which was similar in sterilized and non-sterilized soil at the end of the experiment. However, at this final stage *F. vivipara* in sterilized soil might have benefitted from an inorganic P concentration which was still higher than in non-sterilized soil (Table 2), combined with the benefits of a large system of fine roots developed at an earlier stage (which maximise the exploration of the soil volume), and of retranslocation of leaf nutrients absorbed earlier.

The growth and final biomass of the graminoids in non-sterilized soil was not significantly affected by ex-

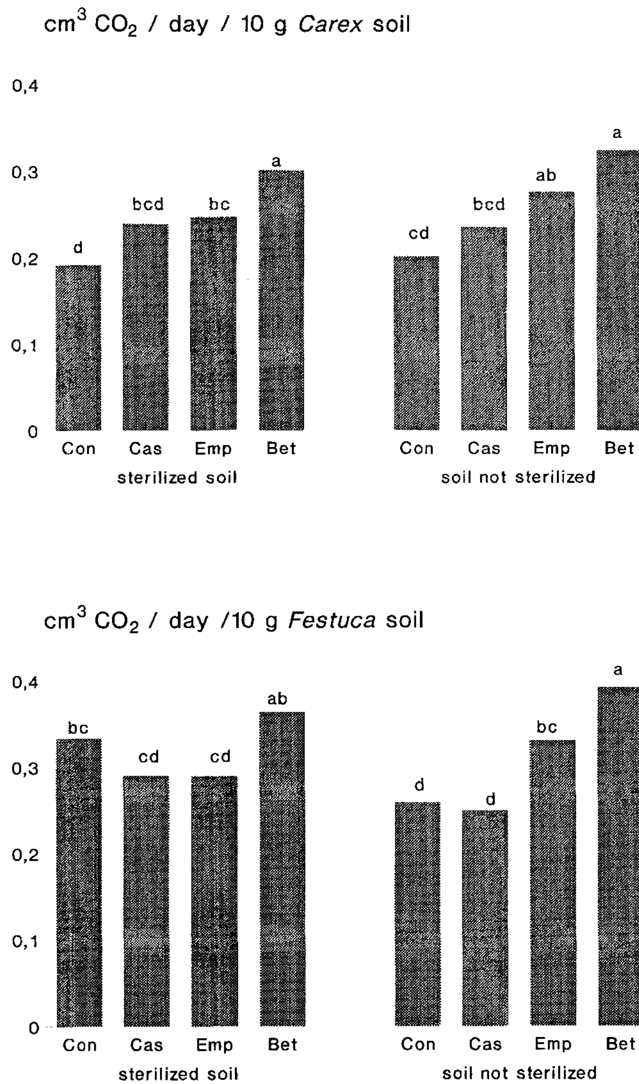

Fig. 5 Microbial respiration in soil from pots with *Carex bigelowii* and *Festuca vivipara* exposed to distilled water or leaf extracts of *Cassiope tetragona* (*Cas*), *Empetrum hermaphroditum* (*Emp*) and *Betula tortuosa* (*Bet*). The means of the eight treatments are compared with Tukey's test at $P = 0.05$; means with same letter are not significantly different ($n = 11$)

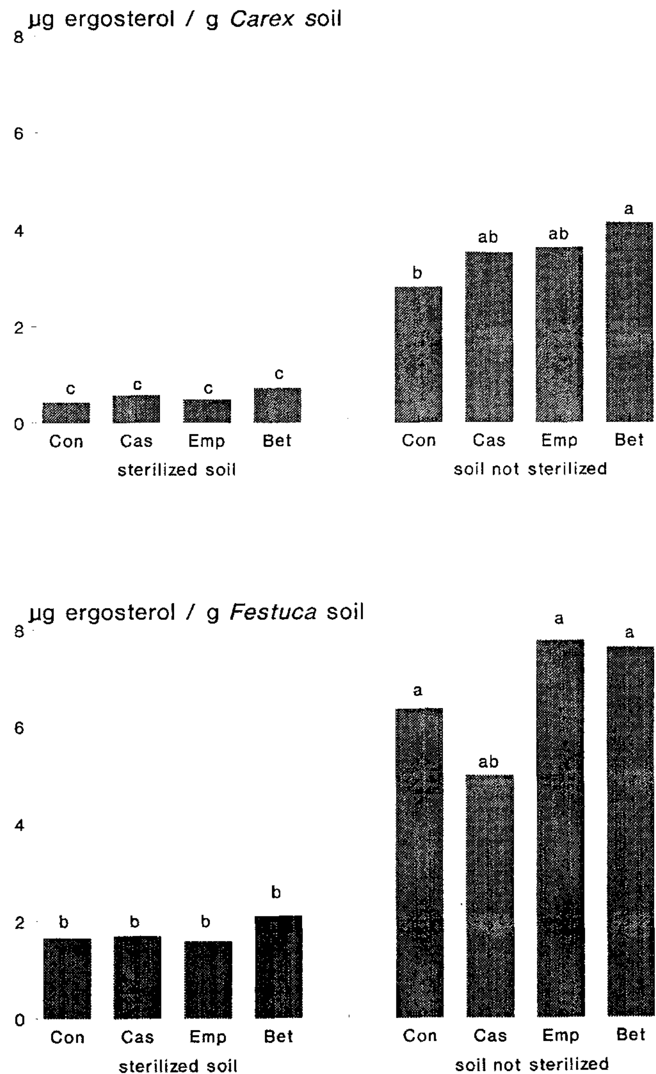

Fig. 6 Ergosterol content of soil from pots with *Carex bigelowii* and *Festuca vivipara* exposed to distilled water or leaf extracts of *Cassiope tetragona* (*Cas*), *Empetrum hermaphroditum* (*Emp*) and *Betula tortuosa* (*Bet*). The means of the eight treatments are compared with Tukey's test at $P = 0.05$; means with same letter are not significantly different ($n = 11$)

posure to the leaf extracts, although there was a tendency for all extracts to retard growth. We attribute this small response to the strong P limitation which reduced the growth of all graminoids so much that any adverse effects of the addition of leaf extracts were difficult to observe. That extract addition did have a negative influence on the plants is, however, shown by the increased uptake rate of N and P by excised roots after addition of the extracts from the dwarf shrubs (Fig. 3), and the strong increase of N uptake after addition of P-rich *Betula* extract. The increased uptake rate is an indication of nutrient limitation in the plants (Harrison and Helliwell 1979; Harrison et al. 1991; Jones et al. 1991; Dighton et al. 1993); this is also reflected in the negative correlation between the N uptake and the N concentration in the shoots of *Carex bigelowii* ($r = -0.333$; $P < 0.05$) and *F. vivipara* ($r = -0.666$; $P < 0.0001$). In contrast to the low, slightly reduced plant growth, the total microbial activity (measured as the soil respiration) was stimulated (Fig. 5), and, for *C. bigelowii*, the soil ergosterol content increased by extract addition (Fig. 6), indicating stimulation of fungal growth by the leaf extracts.

When the soil was sterilized and nutrients were released from the microbial pool (Table 1; Jonasson et al. 1995), however, strong, negative plant growth responses to leaf extract exposure occurred for all species (Figs. 1, 2), and, as for non-sterilized soil, microbial activity increased above that of the controls after recolonizations. Furthermore, as with excised roots of plants grown in non-sterilized soil, nutrient uptake rates increased strongly.

Even though the growth reductions followed previously reported patterns for experiments on phytotoxicity

of leaf compounds (Hytönen 1992; Lisanework and Michelsen 1993; Nilsson et al. 1993; Nilsson 1994), the detailed response was unexpected. Surprisingly, *Betula* extract which had a particular high P content and also a much higher inorganic N content than extracts from the dwarf shrubs, gave the largest growth reduction, in spite of the high P deficiency of the soil. Hence, the expected effect of enhanced growth when *Betula* extract was added was counteracted by some other factors, to the extent of reducing growth more than when the more nutrient-poor extracts of *Cassiope* and *Empetrum* were added. Also, plants grown with *Betula* extracts showed N deficiency symptoms (Figs. 3, 4). Hence, it appears that the addition of P-rich extract led to a decline in plant available soil inorganic N (Table 2).

Although we cannot exclude allelochemicals as a cause of the observed growth reduction, there is strong circumstantial evidence to suggest that carbohydrates in leaf extracts stimulated microbial nutrient uptake and led to strong microbial competition for plant nutrients to the extent of increasing the N and P deficiency of the plants (as shown by the bioassays), thereby reducing plant growth. This is supported by the negative correlation between the biomass production, growth and N concentration of the graminoids in sterilized soil and (1) the polysaccharide content of the leaf extracts added to the soil (Table 1), and (2) the soil microbial respiration at the end of the experiment (Fig. 5).

Furthermore, it appears that the combination of a high P and polysaccharide content in the *Betula* extract (Table 1) has led to a high microbial N uptake, shown by the low NO_3 content of the soils in the *Betula* treatment (Table 2). This, in turn, could explain the high root N demand, indicating N deficiencies of the graminoids (Fig. 3). Hence, we claim that the growth reduction caused by addition of leaf extracts does not necessarily depend on allelochemicals in the extract. It could be an effect of enhanced microbial growth, nutrient immobilization and therefore competition for plant nutrients induced by addition of an energy source to soil micro-organisms in the form of carbohydrates. Note that previous attempts to reduce the amount of allelochemicals by the addition of activated carbon as a phytochemical absorbant to the soil (Zackrisson and Nilsson 1992; Nilsson 1994) cannot separate carbohydrate effects from allelochemical effects because the activated carbon also retains disaccharides and high-molecular-weight carbohydrates (Mattson and Mark 1971).

Our suggestion that resource competition between soil organisms and plants is an alternative or complementary explanation to growth reduction after input of labile carbon to the soil has support from several previous studies. For instance, it is well known from both agricultural systems and natural ecosystems that addition of carbohydrates to the soil can cause a strong depression of plant biomass production (Rutherford and Juma 1992; Harte and Kinzig 1993; Jonasson et al. 1995). It is also realistic to assume that the effect will be very strong in soils with a small pool of labile carbon, particularly if the nutrient pool is also small. Our suggestion that, following the addition of leaf extracts, resource competition between soil microbes and plants plays a major role in plant growth reduction could be tested further by including an additional set of treatments with high nutrient application in future experiments. This would remove the effects of microbial immmobilization of nutrients and reveal possible allelopathic effects.

The extent to which carbohydrate leakage from plants in situ is large enough to cause a noticeable reduction of plant growth is, however, more uncertain. Data on carbohydrate leakage from Antarctic plants to soil given by Roser et al. (1992) and Melick et al. (1994) was, however, of the same order of magnitude per unit soil volume as in our experiment, which suggests that the effect in situ could be comparable to the effect we observed in the potted plants. It is probable that the effect is species-specific. For instance, plants with a high root to shoot ratio and large nutrient stores, such as *Carex bigelowii* (with a root to leaf ratio approximating 2.0, excluding the rhizome, and a root N concentration of 2.1% in controls), are probably less affected than plants with a low root to shoot ratio, such as *L. arcuata* (root: shoot ratio = 0.4), or plants, like *F. vivipara* (with 0.9% N in roots of controls), with small nutrient reserves. It is also likely that some plants can circumvent microbial-imposed nutrient immobilization. This should apply particularly to ericaceous dwarf shrubs, the dominant plant life form in large parts of the Arctic, as they have access to organically fixed nutrient sources through symbiosis with mycorrhizal fungi, which produce proteolytic enzymes (Read 1991; Michelsen et al. 1995). These fungi can decompose organic compounds and transfer the nutrients directly to the host plant, which thereby avoid competition with free-living microbial decomposers.

More work is clearly needed to separate and quantify allelopathy and resource competition in nutrient limited systems. Manipulation of substrate quality and microbial activity by addition of labile carbon, nutrient elements and microbial inhibitors, could, in combination with detailed studies of the direct influence of leaf compounds on plant nutrient uptake and microbial community composition and activity, assist the interpretation of interspecific competition in the field.

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