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Shoot biomass, $\delta^{13}C$, nitrogen and chlorophyll responses of two arctic dwarf shrubs to in situ shading, nutrient application and warming simulating climatic change

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Abstract As climatic change might induce ecophysiological changes in plants which affect their long-term performance, we investigated responses in above-ground biomass, $\delta^{13}C$, nitrogen and chlorophyll of two evergreen arctic dwarf shrubs, *Cassiope tetragona* and *Empetrum hermaphroditum,* to 5 (biomass, N) or 6 years of shading, nutrient application and air/soil warming at a dwarf shrub dominated tree-line heath (450 m a.s.l) and a high altitude fellfield (1100 m a.s.l.) in Swedish Lapland. Warming enhanced the green biomass (equivalent to the last 3–4 years of leaf production) and the ratio of green to brown biomass of *C. tetragona* at the fellfield, and diluted the shoot N concentration. Fertilizer application led to higher shoot N concentration and larger green-to-brown biomass ratio at both sites, and fertilizer application and warming generally had an additive effect on the green biomass. We conclude that both warming and increased soil nutrient availability stimulated the growth of *C. tetragona* at the fellfield whereas at the heath there was a clear increase in production only if enhanced temperature was combined with nutrient application. Across treatments *C. tetragona* at the fellfield had 0.6 ‰ higher $\delta^{13}C$ and 1.4 mg g^{-1} more leaf N, and the soil organic matter $\delta^{13}C$ was 1.0 ‰ higher at the fellfield than at the heath. However, an increase in shoot N concentration with altitude does not necessarily lead to higher $\delta^{13}C$ as no differences in $\delta^{13}C$ were observed when leaf N of the two dwarf shrubs was increased by fertilizer application *C. tetragona* in non-warmed plots had higher $\delta^{13}C$ values than those from warmed plots at the same altitude, which provides the first in situ experimental validation of the theory that temperature partly is responsible for altitudinal trends in plant carbon isotope discrimination. Increased biomass and chlorophyll concentration of *C. tetragona* in warmed plots points to increased assimilation, at least at the fellfield. As the $\delta^{13}C$-based and, therefore, time-integrated estimate of the ratio of CO_2 concentration in the leaf intercellular spaces to that in the atmosphere (C_i/C_a) also increased, warming probably enhanced the stomatal conductance relatively more than the C assimilation, which may be harmful if climatic change leads to reduced soil moisture content and increased plant competition for water. At both sites *C. tetragona* and *E. hermaphroditum* responded to shade by increasing the concentration of shoot N and photosynthetic pigments whereas biomass production (and therefore also net photosynthesis) did not decline. Shade was accompanied by a 0.6–1.3‰ *(E. hermaphroditum)* or 1.2–2.2‰ *(C. tetragona)* decrease in $\delta^{13}C$. This could be due to enhanced stomatal conductance with shading, and perhaps to shade reducing the ericoid mycorrhizal uptake of soil organic C, a factor which has been overlooked as an influence on plant $\delta^{13}C$.

Key words Altitude · Carbon isotopes · *Cassiope tetragona* · *Empetrum hermaphroditum* · Global change

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Introduction

Global change scenarios predict that mean summer air temperature in northern tundra ecosystems may increase by 0.3–1.0° C per decade during the next century (Houghton et al. 1990; Maxwell 1992). The ability of arctic plants to adjust their nutrient and carbon acquisition to the changes while facing competition from neighbouring and immigrating species will have great influence on the future development of arctic plant communities.

Previous research has shown that tundra plants differ widely in their response to changes in the environment and that no common factor limits the growth of all species in such communities (Chapin and Shaver 1985; Parsons et al. 1994). Responses are also strongly site-specific, as growth-limiting factors vary along environmental gradients. For example, in a study of the responses of the ericaceous plant *Cassiope tetragona* to 3 years of nutrient addition, shading and temperature increase, it was concluded that nutrient availability was the main growth-limiting factor near the lower distributional limit of this circumarctic dwarf shrub, whereas temperature was limiting at higher altitudes and latitudes (Havström et al. 1993).

The short-term nature of most of the studies on plant responses to environmental change limits the conclusions which can be drawn from such studies. For example, shade increased the leaf mass of *C. tetragona* (which was surprising because the species never grows in shaded habitats), but this could have been due to internal retranslocation of resources which temporarily compensated for a negative effect of shading during the 3-year study period (Havström et al. 1993). An ecophysiological approach to the study of plant performance in the field can facilitate the understanding of the responses of plants to perturbations. However, many of the existing *in situ* ecophysiological studies on arctic plants exposed to environmental manipulations (Bowman et al. 1993; Baddeley et al. 1994; Welker et al. 1993; Wookey et al. 1994) have been performed after one or two seasons of manipulations only, while long-lived, slow-growing plants may not have acclimatised fully after such a short period.

The aim of the present study was to elucidate how shade, nutrient application and warming of a subarctic heath and a high altitude fellfield over a longer term, 5–6 years, affects the growth of *Cassiope tetragona* and its photosynthetic investment as measured by the concentration of N and photosynthetic pigments. At the heath site we also studied another evergreen, xeromorphic dwarf shrub, *Empetrum hermaphroditum,* which, like *C. tetragona,* is common in heaths and fellfields in the Arctic (Bliss and Matveyeva 1992) but also dominates the field layer in boreal forests (Hultén 1968). *E. hermaphroditum* was included in order to compare the responses of species with similar growth forms and strategies for nutrient acquisition but with presumed differences in their tolerance to shade.

We also used ^{13}C natural abundance ($\delta^{13}C$) analysis to yield a time-integrated measure of the relation between plant stomatal conductance and C assimilation (Farquhar et al. 1989; Ehleringer 1991). When combined with data on plant biomass, nutrient and pigment content, the $\delta^{13}C$ could enable us to predict the longer-term plant responses to environmental change. Plant $\delta^{13}C$ is controlled by the C isotope ratio of the CO_2 source, and the discrimination during plant C assimilation. This discrimination depends on the rate of photosynthesis and the stomatal conductance; hence the relation between $\delta^{13}C$, photosynthetic gas exchange and the ratio of CO_2 concentration in the intercellular spaces to that in the atmosphere (C_i/C_a) (Farquhar et al. 1989; Ehleringer 1991). As environmental conditions, e.g. irradiance, soil moisture and soil nutrient status, affect the C_i/C_a through effects on stomatal opening and rate of photosynthesis, C isotope analysis integrates plant physiological responses to environmental changes, e.g. with an expected increase in $\delta^{13}C$ with decreasing soil moisture.

An additional aim was to reveal the effect of experimental *in situ* temperature change on dwarf shrub C isotope discrimination, to complement earlier investigations from controlled environments (Morecroft and Woodward 1990) and surveys along altitudinal gradients (Körner et al. 1991) showing increased $\delta^{13}C$ with decreasing temperature. Most of the few existing accounts on plant $\delta^{13}C$ in arctic and alpine regions, e.g. those of Körner et al. (1988, 1991) have focused on forbs and graminoids, although dwarf shrubs dominate many arctic plant communities (Bliss and Matveyeva 1992). We anticipated that the extensive environmental perturbations at our well-described (Havström et al. 1993; Jonasson et al. 1993) sites at two altitudes in the subarctic offered an opportunity to investigate the effects of environmental factors and their interactions on $\delta^{13}C$ of two dominant arctic dwarf shrubs.

We included measurements of soil organic matter $\delta^{13}C$ in order to reveal how closely soil and plant $\delta^{13}C$ were correlated in the systems. Soil $\delta^{13}C$ is controlled by the plant litter input and the overall isotopic fractionation during decomposition (Nadelhoffer and Fry 1988; Balasdent et al. 1993), but feedback from soil to vegetation is possible as ericoid mycorrhizal fungi provide their hosts with N, and perhaps C, from organic sources in the soil (Michelsen et al. in press). This could influence plant $\delta^{13}C$, an aspect which has been previously overlooked.

Materials and methods

Experimental manipulations

The samplings took place during the summer of 1993 and 1994 in two plant communities dominated by *Cassiope tetragona* (L.) D. Don at Abisko in northern Swedish Lapland. These communities are located within a subalpine heath close to the tree limit at 450 m above sea level (a.s.l.) and at a fellfield at 1150 m a.s.l., and have been experimentally manipulated since 1989. The climate at the sites is montane subarctic, with a growing season of approximately 3 months, from mid-late June to early-mid September; see Havström et al. (1993), Jonasson et al. (1993) and Michelsen et al. (in press) for more detailed accounts on climate, vegetation and soil parameters at the two sites.

Each year in early June, just after snowmelt, and until the end of August or early September, the temperature and light were manipulated at each site within 6 blocks each of 400 m^2 by erecting dome-shaped plastic greenhouses and shading screens of about 50 cm height and with 1.2×1.2 m surface area. There were two types of greenhouses, one with a 5–10 cm gap above the ground on two sides (T1), and the other with the plastic fixed more tightly to the ground (T2). Both types, consisting of 0.05-mm-thick polyethylene film supported by PVC tubes, had an open top of 40×40 cm in the middle and gave a low and a high temperature increase, respectively. Integrated temperatures were measured in 1991 (air temperature), 1993 and 1994 (soil temperature) with plastic cells

filled with a resin that absorbs water at a temperature-dependent rate (Ambrose 1980). The cells were immersed in distilled water and placed 30 cm above and 3–5 cm below the soil surface, respectively, and left in the field during the growing season (Table 1). For further details on air, soil and *C. tetragona* shoot temperatures see Havström et al. (1993) and Jonasson et al. (1993). Photosynthetically active radiation (PAR) was reduced by 9% inside the greenhouses (Havström et al. 1993).

The shading treatment reduced the light by 64%, which is similar to the reduction in global radiation found within a range of subarctic open forest canopies (Lafleur and Mantha 1994). The shading furthermore resembled the transmittance and optical properties within the range of PAR of an Abisko *Betula pubescens* ssp. *tortuosa* forest with a leaf area index of 2 (Havström et al. 1993). The shading did not change the air humidity (Havström et al. 1993). It was of a similar construction to the greenhouses, but the cover was hessian (sack cloth) instead of polyethylene film.

Table 1 Integrated growing season air and soil temperature (°C) in control plots, plots shaded with hessian and plots with two levels of temperature enhancements at the tree-line heath and the fellfield. Means ± SE, n=3–4. Data on integrated air temperature are from Jonasson et al. (1993). For full details on diurnal variations of shoot and air temperatures in 1991, see Havström et al. (1993)

	Air temperature (°C)	Soil temperature (°C)	
	1991	1993	1994
Tree-line heath			
Control	10.9±0.03	7.1±0.13	6.7±0.21
Shaded	11.0±0.05	7.8±0.27	6.3±0.06
Temperature 1	13.7±0.12	7.7±0.75	7.1±0.60
Temperature 2	14.8±0.46	8.3±0.36	8.5±0.35
Fellfield			
Control	6.8±0.09	5.1±0.15	6.0±0.34
Shaded	6.9±0.10	5.1±0.07	4.7±0.71
Temperature 1	9.2±0.19	5.7±0.22	8.0±0.49
Temperature 2	11.7±0.07	6.4±0.13	7.2±0.16

The experiments were done in sloping terrain which permitted soil water to move laterally into the plots. The water content of the soil, which was measured gravimetrically on soil samples taken from 0–10 cm depth at the heath and 0–3 cm depth at the fellfield at three occasions in 1993 and at two occasions in 1994, was therefore unaffected by both the temperature and the shade treatments (Fig. 1).

A factorial fertilizer treatment, simulating increased net nutrient mineralisation if climate changes, was included in the experiment, in which 10 g m^{-2} N, 2.6 g m^{-2} P and 9 g m^{-2} K (4.9, 1.3 and 6.0 g m^{-2} N, P and K in 1989) was added in June in all years except 1993. This treatment enhanced the N and P in the soil inorganic and microbial pools (Michelsen A., Jonasson S., Sleep D., Schmidt I.K., Callaghan T.V., unpublished work). Hence, each block contained one unfertilized and one fertilized set of shading, low- and high-temperature enhancement treatments, and fertilized and unfertilized controls, i.e. eight treatments altogether, replicated over six blocks.

Aboveground plant biomass harvest

On 21 July (heath) and 23 July (fellfield) 1993 all above-ground vegetation inside a randomly chosen area of 20×20 cm was sampled in each plot.

Branches of *C. tetragona* and *E. hermaphroditum* Hagerup were separated from the total phytomass of the tree-line heath. *C. tetragona* alone was separated from the fellfield samples as *E. her-*

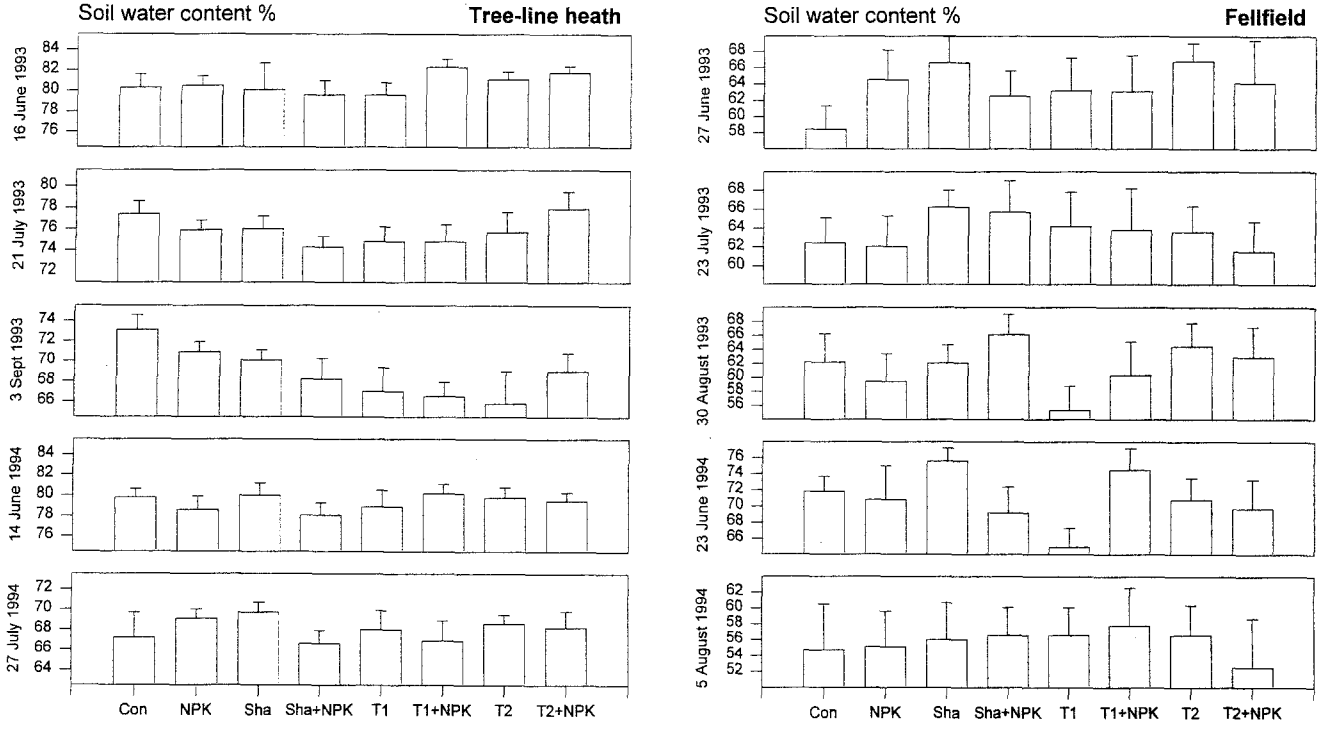

Fig. 1 Early, mid and late season soil water content (%) at the tree-line heath and the fellfield (note that the y axis scale does not begin at 0%). The treatments were: controls (*Con*), shading with hessian (*Sha*), and two levels of temperature enhancements (*T1* and *T2*), all with and without NPK fertilizer application. Means ± SE, n=6. Sampling dates at the heath were 16 June, 21 July and 3 September 1993, and 14 June and 27 July 1994. At the fellfield soil was sampled 27 June, 23 July and 30 August 1993, and 23 June and 5 August 1994. ANOVAs with temperature manipulation, fertilizer application and light as main effects were non-significant at all sampling times at both sites, except for reduced soil water content with temperature manipulations at the heath in September 1993 (F=4.95, P=0.0154)

maphroditum was rare at this site. *C. tetragona* branches were separated in a green part, equivalent to the last three to four years of leaf growth (Callaghan et al. 1989; Havström et al. 1993), and a brown part which included branches with and without yellow senescing and brown, dead leaves. The material was dried at 80° C to constant weight, and each fraction was weighed. The material was ground in a Tecator Cyclotec 1093 Sample Mill for subsequent analysis of $\delta^{13}C$, C and N concentration.

Soil collection and preparation

Within each plot, soil was collected randomly with a corer as five 10-cm (heath) and 3-cm (fellfield) deep plugs of humus, on 3 September 1993 and 28 August 1993, respectively. After sampling the soil was kept refrigerated at 4° C. The five samples from each plot were bulked, and pieces of mosses, coarse and fine roots were removed from the soil within four days after collection, after which the soil was frozen. A 10-g subsample of this soil was dried at 70° C, ground in a freezer mill and subsequently analysed for $\delta^{13}C$.

Photosynthetic pigment analysis

Shoots of *C. tetragona* for leaf pigment analysis were collected at noon 1 August (heath) and 2 August (fellfield) 1994, in full sunlight. Shoots of *E. hermaphroditum* were collected on 4 August 1994, also in full sunlight. Ten shoots were randomly selected from each plot, placed in sealed plastic bags and were kept cool and in the dark until analysis 3–5 h later.

Ten *(C. tetragona)* and 15 *(E. hermaphroditum)* of the youngest, fully expanded leaves (i.e. of last years cohort) were taken distally on the shoot tips, with one to two leaves from each shoot. The leaves were weighed and quickly ground in a mortar with 80% acetone. The sample was centrifuged at 3500 rpm for 10 min., brought up to 5 ml in volumetric flasks and measured in a Shimadzu spectrometer UV-160A at 663.2, 646.8 and 470 nm for chlorophyll *a,* chlorophyll *b* and carotenoids (Lichtenthaler 1987). Equivalent leaf samples were weighed and dried for dry weight conversion factors.

Analysis of $\delta^{13}C$ and N concentration

Samples of about 3 mg dried, ground green plant material of *C. tetragona,* and dried, ground whole-shoot material of *E. hermaphroditum* from the biomass harvest of 1993 were put into preweighed tin capsules, redried at 105° C, cooled, crimped, reweighed and analysed for $\delta^{13}C$ and C concentration. The analyses were carried out on a Roboprep sample converter interfaced with a Tracermass isotope ratio mass spectrometer (Europa Scientific Ltd, Crewe, UK) (Knight et al. 1994). Soil $\delta^{13}C$ was measured on samples of approximately 2.8 mg (heath) and 4.5 mg (fellfield), both equivalent to 1.0 mg C. The sample sizes of plant and soil were adjusted to yield the same amount of C as in the standard material (Michelsen and Sprent 1994). The precision of the $\delta^{13}C$ analyses was better than 0.2‰.

The analysis of the N concentration in *E. hermaphroditum* and in green parts of *C. tetragona* was performed on 10-mg plant samples using the same methods and equipment as above, whereas the brown parts of this species were analysed for N concentration by the salicylate method (Kedrowski 1983) using a Hitachi U-2000 spectrophotometer after acid digestion.

Carbon isotope results are reported in ‰: $\delta^{13}C=1000\times(R_{sample}-R_{standard})/R_{standard}$ (‰), where $R=^{13}C/^{12}C$, and the working standard was beet sucrose material calibrated against Pee Dee Belemnite; it is convenient to use the $\delta^{13}C$ notation when linking studies of leaf and soil carbon dynamics. We calculate the ratio of CO_2 in the leaf intercellular spaces to that in the atmosphere (C_i/C_a) based on the leaf carbon isotope discrimination $\Delta=(\delta^{13}C_{air}-\delta^{13}C_{leaf})/(1-\delta^{13}C_{leaf})$, where we assume an $\delta^{13}C_{air}$ of –8.0‰ (Farquhar et al. 1989). C_i/C_a then equals $(\Delta-a)/(b-a)$, where a is the discrimination factor associated with stomatal diffusion of CO_2 (4.4‰) and b is the estimated discrimination factor associated with carboxylation (–27.0‰) (Farquhar et al. 1989).

Statistical analysis

Statistics were performed using the SAS package (SAS Institute 1988) throughout. The experiment had two parts, one assessing light/nutrient effects and one assessing temperature/nutrient effects on the plants, each in a factorial design. Hence, each parameter was analysed with two separate ANOVAs: one with temperature manipulation (three levels) and fertilizer application (two levels) as main effects, and one with light (two levels) and fertilizer application (two levels) as main effects. All samplings and analyses were done in blocks. A few plots completely lacked *E. hermaphroditum* and were *a priori* removed from the analyses; hence the number of replicates of this species was five for the combined fertilizer and shading treatment, and four for the combined fertilizer and high temperature treatment.

Results

Aboveground biomass of C. tetragona and E. hermaphroditum

C. tetragona was the dominant plant species at the two sites. It constituted more than one-third of the total above-ground biomass at the heath and more than one-half at the fellfield. The total mean above-ground biomass in unperturbed plots was 1000 g m^{-2} at the heath site, and 380 g m^{-2} at the higher altitude fellfield; of this amount 405 g m^{-2} and 215 g m^{-2}, respectively, were *C. tetragona* (Fig. 2).

The temperature and nutrient manipulated plots had higher green biomass of *C. tetragona* at both sites (Fig. 2, Table 2), but the ratio of green-to-brown biomass did not increase in temperature enhanced plots at the heath. Although the biomass was higher in warmed heath plots, the unchanged ratio of green to brown plant parts might indicate that the growth was little affected by temperature and that the difference in biomass was because a few quadrats with very high biomass happened to be selected for harvests in the high temperature treatments. The green biomass (Table 2) and the green-to-brown biomass ratio in shaded plots were not significantly different from the controls, nor were there any significant differences in biomass of the fraction of *C. tetragona* with brown leaves plus stems in any of the treatments (ANOVAs not presented).

At the heath *E. hermaphroditum* constituted 5–20% of the above-ground plant biomass and was not regularly distributed across the site giving a strong block effect in the ANOVA (Table 2). No significant biomass effects of the perturbations could be assessed with this species; the relatively large standard errors indicate that the sample area (20 cm×20 cm) was too small to yield a biomass estimate of high precision for a less common species with the number of replicates we used. *E. hermaphroditum* was rare at the fellfield and was therefore not sampled here.

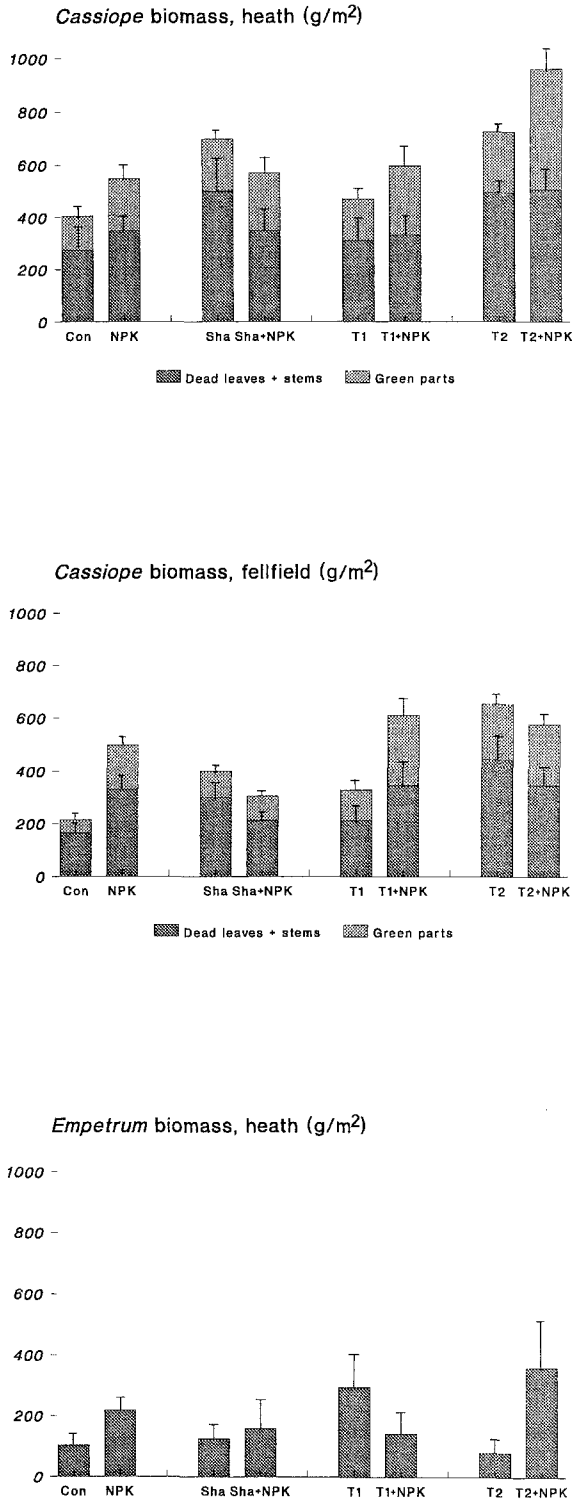

Fig. 2 Biomass of *Cassiope tetragona* and *Empetrum hermaphroditum* in tree-line heath and *C. tetragona* in fellfield, harvested 21 and 23 July 1993. The treatments were: controls (*Con*), shading with hessian (*Sha*), and two levels of temperature enhancement (*T1* and *T2*), all with and without NPK fertilizer application. Means ± SE, n=6, except for *E. hermaphroditum* which has n=(4–)6

Concentration of plant nitrogen and photopigments

The N concentration in green parts of *C. tetragona* across treatments was higher at the fellfield (13.2±0.3 mg g^{-1} N) than at the heath (11.8±0.2 mg g^{-1} N) (t test: t=–3.8846, P=0.0002, df=94). Light, temperature and nutrient manipulations affected the shoot N concentration of *C. tetragona* in a similar way at the two sites (Tables 3 and 4). Shaded and fertilized *C. tetragona* had higher N concentration in both above-ground components, whereas the N concentration in warmed plots was slightly lower in the green parts in the low temperature enhancement at the heath and much lower in the two biomass components in both temperature treatments at the fellfield. When temperature enhancement and fertilizer application was combined, the N concentration in the green part was not significantly different from the controls whereas it was higher than controls in the non-green part. Combined shading and fertilizer application had an additive effect on N concentration.

The N concentration in *E. hermaphroditum* was lower than in *C. tetragona* and declined continuously as the temperature increased from the control to the high temperature enhancement treatment. Also in the response to shading and nutrient addition the pattern was similar to that of *C. tetragona* at the fellfield (Table 5). *E. hermaphroditum* had a lower pigment content per dry mass of leaf then *C. tetragona* (Fig. 3). The pigment content of the latter species was similar at the heath and the fellfield. In general, the response to the manipulations were similar for chlorophyll *a* and *b* (Fig. 3) and carotenoids (not presented) but the response was least for carotenoids and proportionally strongest for chlorophyll *b*. In response to shading, *E. hermaphroditum* and *C. tetragona* strongly enhanced their leaf chlorophyll content (Fig. 3, Table 6). Furthermore, the chlorophyll *a/b* ratio of *C. tetragona* was reduced by shading. Temperature or fertilizer manipulations did not affect the chlorophyll content of *E. hermaphroditum*. Fertilizer application enhanced the chlorophyll content of *C. tetragona* at the fellfield, and temperature increased the content at the heath. The N concentration and the total chlorophyll concentration of *C. tetragona* were correlated both at the heath (Pearson correlation coefficient r=0.4970, P<0.001) and at the fellfield (r=0.6537, P<0.0001).

Plant and soil $\delta^{13}C$

The average $\delta^{13}C$ of *C. tetragona* was –27.52±0.09‰ at the high altitude fellfield and –28.14±0.14 at the heath, i.e. a difference of 0.6‰ between the two sites (*t*-test: t=–3.56, P=0.0006, df=94). The responses to the treatments were similar both between the two sites and the two species (Tables 3 and 5). In all cases the $\delta^{13}C$ was more negative in shaded plants, by up to 2.2‰. Hence the ratio of CO_2 concentration in the leaf intercellular spaces to that of the atmosphere (C_i/C_a) was closer to unity for shaded plants (Fig. 4). Fertilizer application did not af-

Table 2 ANOVAs on biomass of green parts of *Cassiope tetragona* in tree-line heath and fellfield, harvested 21 and 23 July 1993, and on total above-ground biomass of *Empetrum hermaphroditum* in tree-line heath, harvested 23 July 1993

	df	*F*	*P*		*df*	*F*	*P*
C. tetragona green biomass, tree-line heath							
Block	5	0.62	0.6870	Block	5	0.23	0.9468
Shade	1	0.89	0.3607	Temperature	2	3.74	0.0380
Fertilizer	1	0.95	0.3449	Fertilizer	1	5.85	0.0232
Shad×fert	1	0.24	0.6338	Temp×fert	2	0.73	0.4909
C. tetragona green biomass, fellfield							
Block	5	1.66	0.2051	Block	5	0.53	0.7504
Shade	1	0.37	0.5525	Temperature	2	3.87	0.0342
Fertilizer	1	8.00	0.0127	Fertilizer	1	8.02	0.0090
Shad×fert	1	10.39	0.0057	Temp×fert	2	1.34	0.2802
E. hermaphroditum total above-ground biomass, tree-line heath							
Block	5	7.93	0.0008	Block	5	3.94	0.0091
Shade	1	0.17	0.6851	Temperature	2	0.32	0.7277
Fertilizer	1	2.61	0.1273	Fertilizer	1	1.39	0.2501
Shad×fert	1	0.77	0.3947	Temp×fert	2	3.39	0.0499

Table 3 Nitrogen concentration in green and brown above-ground biomass component, and $\delta^{13}C$ in green leaves of *C. tetragona* in tree-line heath and fellfield, harvested 21 and 23 July 1993, and $\delta^{13}C$ in soils sampled August 1993. The treatments were: controls, shading with hessian, and two levels of temperature enhancements, all with and without NPK fertilizer application. Means ± SE, *n*=6; *n.d.* not determined

	Dead leaves and stems	Green leaves		Soil
	Nitrogen (mg g^{-1})	Nitrogen (mg g^{-1})	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)
Tree-line heath				
Control	6.8±0.3	11.2±0.6	−27.29±0.18	−26.50±0.14
NPK fertilizer	9.0±0.8	12.4±0.5	−27.46±0.20	−26.64±0.16
Shaded	8.4±0.5	12.7±0.4	−29.13±0.10	−26.60±0.19
Shaded+NPK	10.9±0.8	13.9±0.5	−29.71±0.36	−26.75±0.23
Temperature 1	6.5±0.4	10.1±0.3	−27.64±0.23	−26.15±0.14
Temperature 1+NPK	8.7±0.8	11.9±0.4	−28.39±0.25	−26.94±0.15
Temperature 2	6.8±0.3	10.9±0.5	−28.02±0.27	−26.48±0.13
Temperature 2+NPK	8.2±0.4	11.2±0.4	−27.52±0.31	−26.74±0.12
Fellfield				
Control	9.7±0.3	12.5±0.3	−26.92±0.16	−25.51±0.17
NPK fertilizer	11.1±0.5	13.7±0.3	−27.17±0.19	n.d.
Shaded	10.7±0.8	14.1±0.7	−28.46±0.15	−25.81±0.10
Shaded+NPK	12.5±0.6	16.7±0.6	−28.35±0.24	n.d.
Temperature 1	9.4±0.5	11.6±0.4	−27.51±0.04	n.d.
Temperature 1+NPK	10.4±0.4	12.5±0.3	−26.96±0.23	n.d.
Temperature 2	8.6±0.3	10.9±0.3	−27.48±0.14	n.d.
Temperature 2+NPK	10.2±0.3	13.2±0.2	−27.34±0.18	n.d.

fect the $\delta^{13}C$ whereas warming slightly reduced the $\delta^{13}C$ signatures in the case of *C. tetragona*. However, in combination with fertilizer application this effect of temperature manipulations seemed to diminish, evident as the temperature × fertilizer interactions in the ANOVAs (Table 4). In general the within-treatment variation was higher for *E. hermaphroditum* and the responses smaller, perhaps because whole-shoot samples were used for this species.

The average soil $\delta^{13}C$ was −25.65±0.11‰ at the high altitude fellfield and −26.60±0.06 at the heath, i.e. there was a 1.0‰ difference between the two sites (*t*-test: t=−6.92, P<0.0001, df=56). In contrast to the plant $\delta^{13}C$, shading or temperature manipulation did not affect the soil $\delta^{13}C$, whereas fertilizer application reduced the soil $\delta^{13}C$ slightly (Tables 3 and 4).

The shoot and soil C concentration did not differ between treatments.

Discussion

Biomass, nitrogen and photosynthetic pigment responses to the perturbations

The longer-term (5 years) biomass responses of the two dwarf shrubs to fertilizer application and temperature increase (Fig. 2, Table 2) are coherent with the vegetative responses recorded for *C. tetragona* at the same sites after 3 years of manipulations (Havström et al. 1993), and with those of *E. hermaphroditum* in a nearby mountain birch forest after one (Wookey et al. 1993) and two (Parsons et al. 1994) seasons, respectively. Similarly to Parsons et al. (1994), we did not observe significant effects on *E. hermaphroditum* biomass, but the sensitivity of the test was weak due to rather large variance among replicates. The nutrient dilution after warming and the increase in nutrient concentration in the shoots after NPK application

Table 4 ANOVAs on N concentration in green and brown above-ground biomass component, and on $\delta^{13}C$ of green leaves of *C. tetragona* and of soil at tree-line heath and fellfield. Effect of shading on soil $\delta^{13}C$ was tested with *t*-test at the fellfield: t=1.45, P=0.1808

	df	*F*	*P*		*df*	*F*	*P*
N concentration in green part, tree-line heath							
Block	5	0.55	0.7348	Block	5	5.24	0.0020
Shade	1	7.25	0.0167	Temperature	2	3.69	0.0393
Fertilizer	1	5.44	0.0341	Fertilizer	1	16.47	0.0004
Shad×fert	1	0.01	0.9280	Temp×fert	2	2.65	0.0903
N concentration in brown part, tree-line heath							
Block	5	1.76	0.1817	Block	5	0.60	0.7024
Shade	1	8.51	0.0106	Temperature	2	0.30	0.7436
Fertilizer	1	15.46	0.0013	Fertilizer	1	17.93	0.0003
Shad×fert	1	0.08	0.7867	Temp×fert	2	0.28	0.7609
N concentration in green part, fellfield							
Block	5	1.07	0.4152	Block	5	0.26	0.9282
Shade	1	21.10	0.0004	Temperature	2	6.61	0.0050
Fertilizer	1	15.56	0.0013	Fertilizer	1	26.72	0.0001
Shad×fert	1	2.18	0.1603	Temp×fert	2	2.48	0.1042
N concentration in brown part, fellfield							
Block	5	0.42	0.8256	Block	5	0.43	0.8215
Shade	1	3.64	0.0759	Temperature	2	3.36	0.0508
Fertilizer	1	5.81	0.0292	Fertilizer	1	15.34	0.0006
Shad×fert	1	0.12	0.7341	Temp×fert	2	0.30	0.7461
$\delta^{13}C$, tree-line heath							
Block	5	0.72	0.6174	Block	5	3.17	0.0236
Shade	1	72.31	0.0001	Temperature	2	4.68	0.0188
Fertilizer	1	2.44	0.1388	Fertilizer	1	0.71	0.4062
Shad×fert	1	0.71	0.4128	Temp×fert	2	4.44	0.0224
$\delta^{13}C$, fellfield							
Block	5	3.02	0.0440	Block	5	2.15	0.0927
Shade	1	77.91	0.0001	Temperature	2	2.81	0.0791
Fertilizer	1	0.21	0.6545	Fertilizer	1	1.38	0.2506
Shad×fert	1	1.38	0.2587	Temp×fert	2	3.44	0.0480
Soil $\delta^{13}C$, tree-line heath							
Block	5	0.75	0.5997	Block	5	0.39	0.8484
Shade	1	0.36	0.5571	Temperature	2	0.12	0.8891
Fertilizer	1	0.55	0.4696	Fertilizer	1	11.50	0.0024
Shad×fert	1	0.01	0.9359	Temp×fert	2	2.75	0.0843

show that this species does indeed respond to changes in temperature and nutrients, as also shown by productivity measurements below (Parsons et al. 1994) and above the tree-line, at our sites (nutrients only; Graglia E., Jonasson S., Michelsen A., Schmidt I.K., unpublished work). This also agrees with observations in Alaskan tussuck tundra (Chapin and Shaver 1985).

The much enhanced green biomass production of *C. tetragona* at the fellfield caused by warming did not lead to a proportional dilution of the shoot N concentration; hence *C. tetragona* can at least partly adjust its nutrient uptake to the growth. The effect of warming on *C. tetragona* was less at the heath, possibly because warming generally does not stimulate the growth of high Arctic species at their lower altitudinal distribution limit. The slight increase in soil inorganic N concentration by warming (Michelsen A., Jonasson S., Sleep D., Schmidt I.K., Callaghan T.V., unpublished) did not affect the growth of *C. tetragona*; evergreen species in other N limited habitats similarly show low growth response to small increases in the N supply as the response curve is exponential (Schulze et al. 1994).

Fertilizer application led to higher shoot N concentration and larger biomass. With the assumption that montane sub- and high-Arctic populations of the species show similar photosynthetic reponses to fertilizer application the larger biomass is probably because the enhanced nutrient availability allowed *C. tetragona* to attain maximum rates of C assimilation earlier in the season (Baddeley et al. 1994). The increased growth after fertilizer application and after warming confirms that both soil nutrients and temperature can limit its growth (Callaghan et al. 1989; Havström et al. 1993) but that responses could be site specific. At the heath, Havström et al. (1993) reported responses to fertilizer addition after 3 years of treatment, but not to raised temperature, which is similar to our data after 5 years of treatment if we interpret our data on the response of the biomass fractions conservatively. At the fellfield, Havström et al. (1993) found effects of temperature enhancement only, while we found also growth responses to nutrient addition. This difference could be due to either a difference in response after 3 and 5 years of nutri-

Table 5 Nitrogen concentration and $\delta^{13}C$ in above-ground parts of *E. hermaphroditum* in tree-line heath harvested 23 July 1993. The treatments were: controls, shading with hessian, and two levels of temperature enhancements, all with and without NPK fertilizer application. Means±SE, n=(4–)6

	Nitrogen (mg g^{-1})	$\delta^{13}C$ (‰)
Control	6.9±0.1	−27.25±0.62
NPK fertilizer	7.6±0.3	−28.18±0.50
Shaded	7.3±0.3	−28.55±0.36
Shaded+NPK	9.7±0.8	−28.83±0.97
Temperature 1	6.3±0.3	−27.20±0.47
Temperature 1+NPK	7.3±0.5	−28.39±0.51
Temperature 2	5.6±0.2	−28.26±0.19
Temperature 2+NPK	6.3±0.3	−27.38±0.31

ANOVAs	*df*	*F*	*P*	*F*	*P*
Block	5	0.77	0.5902	4.69	0.0154
Shade	1	4.32	0.0597	5.18	0.0438
Fertilizer	1	9.91	0.0084	1.93	0.1925
Shad×fert	1	2.05	0.1780	0.00	0.9705
Block	5	0.77	0.5838	5.89	0.0019
Temperature	2	7.27	0.0042	0.13	0.8793
Fertilizer	1	8.70	0.0079	1.09	0.3091
Temp×fert	2	0.13	0.8757	5.63	0.0120

ent addition, or that new meristems developed as side-branches (Graglia E., Jonasson S., Michelsen A., Schmidt I.K., unpublished) that were not included in the analysis after 3 years which measured responses on main branches only.

Both dwarf shrub species responded to shade by allocating more N to the green leaves and both species also strongly increased the concentration of photosynthetic pigments. Since biomass production did not decline, these changes probably led to unchanged or higher photosynthesis per unit leaf mass despite reduced light intensity (Osmond 1987; Morecroft et al. 1992; Bowman et al. 1993). The ability of *C. tetragona* to acclimatise to shade is somewhat surprising as this species never grows in shaded habitats, as for example subalpine mountain birch *(Betula tortuosa)* forests (Havström et al. 1993). Havström et al. (1993) report similar unchanged leaf biomass production after three years of treatment, but the flowering frequency of this species declined strongly (Havström et al. in press). Thus, reduction of light by as much as 64% apparently does not limit the vegetative growth of *C. tetragona*, although it limits the reproduction.

The ability of *E. hermaphroditum* to acclimatise to shade is less surprising as this species is common not only in tundras but also in boreal forests (Hultén 1968) and mountain birch forests (Sonesson and Lundberg 1974), and hence would be expected to be better adapted to shade than *C. tetragona*. However, Chapin and Shaver (1985) observed reduced growth of *E. hermaphroditum* after 2 years of shading of Alaskan tundra, indicating site specific or genotypic differences in responses.

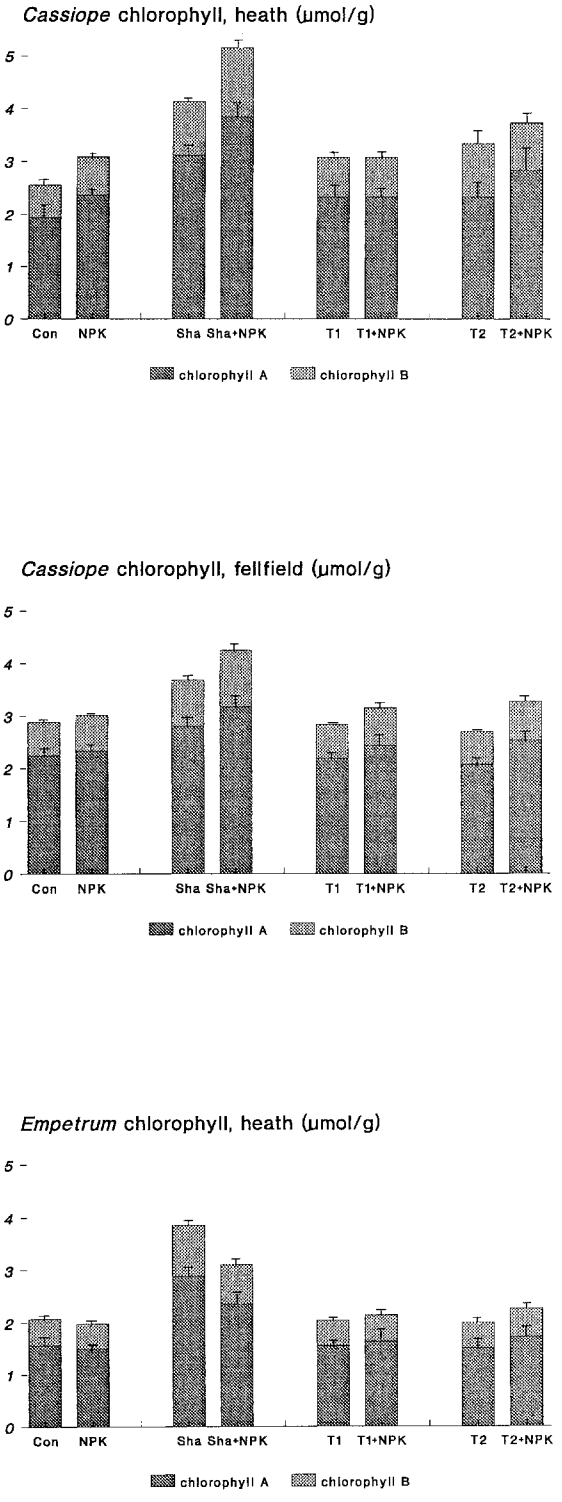

Fig. 3 Chlorophyll A and B in leaves of *C. tetragona* and *E. hermaphroditum* in tree-line heath and *C. tetragona* in fellfield, analysed 1, 2 and 4 Aug 1994. The treatments were: controls (*Con*), shading with hessian (*Sha*), and two levels of temperature enhancements (*T1* and *T2*), all with and without NPK fertilizer application. Means ± SE, n=6, except for *E. hermaphroditum* which has n=(4–)6

Table 6 ANOVAs on total chlorophyll of leaves of *C. tetragona* and *E. hermaphroditum* from tree-line heath and fellfield, and *C. tetragona* from fellfield

	df	*F*	*P*		*df*	*F*	*P*
C. tetragona chlorophyll, tree-line heath							
Block	5	0.29	0.9136	Block	5	3.07	0.0271
Shade	1	55.89	0.0001	Temperature	2	3.35	0.0501
Fertilizer	1	10.04	0.0064	Fertilizer	1	1.81	0.1911
Shad×fert	1	1.01	0.3302	Temp×fert	2	0.47	0.6307
C. tetragona chlorophyll, fellfield							
Block	5	1.12	0.3897	Block	5	1.19	0.3408
Shade	1	32.52	0.0001	Temperature	2	0.04	0.9603
Fertilizer	1	3.87	0.0679	Fertilizer	1	6.50	0.0173
Shad×fert	1	1.51	0.2382	Temp×fert	2	0.94	0.4038
E. hermaphroditum chlorophyll, tree-line heath							
Block	5	1.16	0.3773	Block	5	1.73	0.1671
Shade	1	32.57	0.0001	Temperature	2	0.22	0.8033
Fertilizer	1	2.39	0.1441	Fertilizer	1	0.59	0.4513
Shad×fert	1	1.63	0.2230	Temp×fert	2	0.91	0.4159

Effects of altitude on plant $\delta^{13}C$

The $\delta^{13}C$ of *C. tetragona* across treatments was 0.6‰ higher at the fellfield than at the heath, corresponding to an altitude increase of 700 m. The increased plant $\delta^{13}C$ with altitude corresponds with laboratory experiments and field surveys (Körner et al. 1988, 1991; Morecroft and Woodward 1990; Morecroft et al. 1992; Marshall and Zhang 1994). The $\delta^{13}C$ of *C. tetragona* leaf cellulose from a high Arctic site (Welker et al., in press) (with lower temperature and higher atmospheric pressure than our sites) was higher than the *C. tetragona* $\delta^{13}C$ of our sites. Our perturbations did not alter the relationship between altitude and $\delta^{13}C$; the $\delta^{13}C$ were always highest at the fellfield, when plants of identical treatments from high and low altitudes were compared. The increase in $\delta^{13}C$ with altitude corresponded to an increase in leaf N, as in Morecroft et al. (1992). However, increases in N concentration do not necessarily lead to higher plant $\delta^{13}C$; we did not observe any difference in $\delta^{13}C$ for the two dwarf shrubs when fertilised, although their tissue N increased.

Effects of warming and fertilizer application on plant $\delta^{13}C$

C. tetragona in warmed plots had more negative $\delta^{13}C$ values than in non-warmed plots at the same altitude, both at the heath and at the fellfield; the $\delta^{13}C$ decreased by 0.35 (low temperature enhancement; T1) and 0.73 (high temperature enhancement; T2) ‰ at the heath and by 0.59 (T1) and 0.56 (T2) ‰ at the fellfield, with corresponding air temperature increases of 2.8, 3.9, 2.4 and 4.9° C, compared to controls. A similar response to temperature increase was observed for the montane grass *Nardus stricta* under controlled experimental conditions (Morecroft and Woodward 1990). The results from our *in situ* experiments thus lend support to the suggestion by Morecroft and Woodward (1990) and by Körner et al. (1991) that temperature (as well as atmospheric pressure) is responsible for altitudinal trands in plant carbon isotope discrimination; a comparison of our data on altitudinal and warming effects on $\delta^{13}C$ shows that the temperature component probably is more important than the pressure component.

The temperature effect on $\delta^{13}C$ probably works partly through temperature effects on Rubisco carbon isotope fractionations and partly through anatomical and physiological routes, e.g. due to increased leaf thickness in cold environments (Körner et al. 1991), and by temperature effects on transpiration. In our study the leaf dry weight was unaffected by temperature increase (unpublished data). However, after 2 years of perturbations, the ratio of leaf dry weight to length was higher, not lower, in warmed plots (Havström et al. 1993). The decrease in $\delta^{13}C$ of *C. tetragona* with temperature increase or decrease in altitude thus seems unrelated to leaf thickness.

The effect of temperature increase on the $\delta^{13}C$ and the ratio of CO_2 concentration in the leaf intercellular spaces to that of the atmosphere (C_i/C_a) of *C. tetragona* is similar to that of *Dryas octopetala* in a high arctic environment (Welker et al. 1993) in which identical greenhouses were used, although in their case the leaf biomass was reduced by warming. It is unlikely that the slightly lower tissue N concentration of *C. tetragona* in warmed plots led to a reduction in the C assimilation (which would have contributed to the decreased $\delta^{13}C$). Increased plant biomass and chlorophyll concentration in warmed plots points to increased C assimilation, and data on the photosynthetic rate of herbs from warmed plots of similar construction to ours (Wookey et al. 1994) also supports this. Thus, warming probably reduced the water use efficiency of *C. tetragona* by increasing stomatal conductance relatively more than the C assimilation. This may be beneficial for plants in environments where water does not often limit growth, as it is likely for our sites, but it may be harmful if climatic change leads to reduced soil moisture content and enhanced plant competition for water.

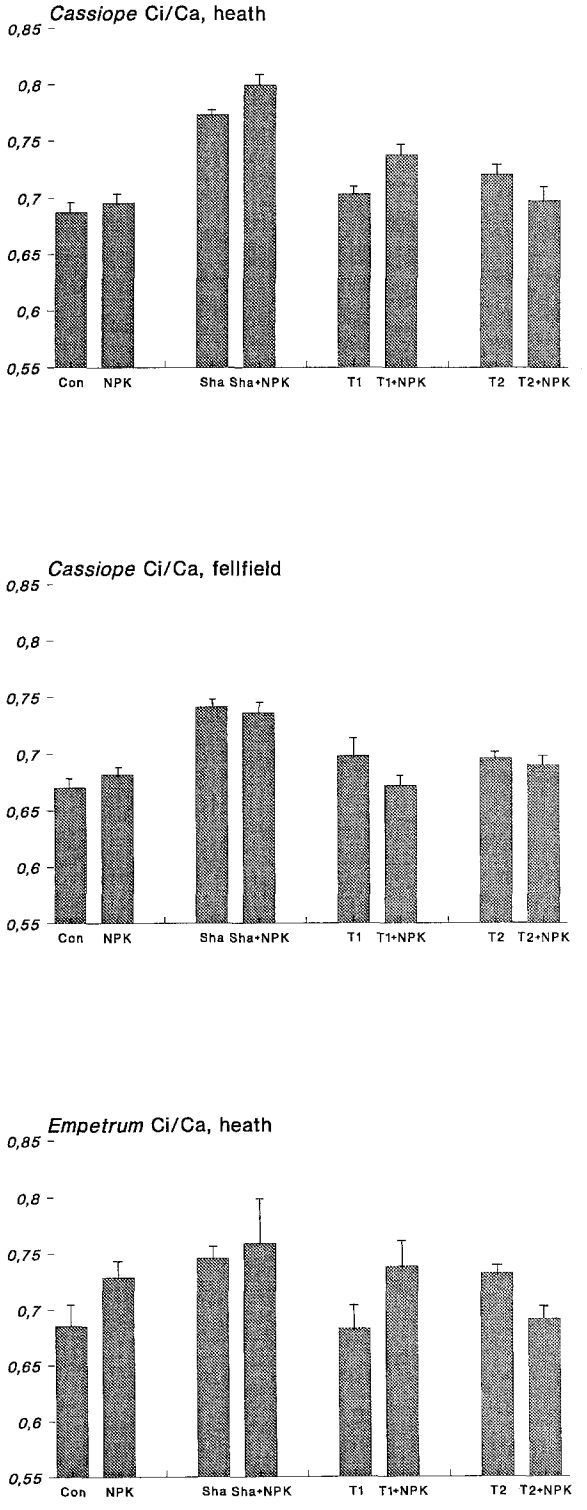

Fig. 4 The ratio of CO_2 concentration in the leaf intercellular spaces to that in the atmosphere (C_i/C_a), for leaves of *C. tetragona* and *E. hermaphroditum* in tree-line heath and *C. tetragona* in fellfield, based on $\delta^{13}C$ analysis of plant material harvested 21 and 23 July 1993. The treatments were: controls (*Con*), shading with hessian (*Sha*), and two levels of temperature enhancements (*T1* and *T2*), all with and without NPK fertilizer application. Means ± SE, n=6, except for *E. hermaphroditum* which has n=(4–)6

The performance of plants to which fertilizer was applied suggests that water did not limit the growth of *C. tetragona* at the heath or the fellfield. Both the aboveground biomass and the N concentration of *C. tetragona* increased, but this did not lead to decreased shoot $\delta^{13}C$, although this would have been the expected response of N-limited plants if their demand for water increased due to fertilizer application (Högberg et al. 1993). Furthermore, in nearby *Betula tortuosa* forest, water application did not enhance the growth of dwarf shrubs (Parsons et al. 1994). Note that our warmed plots generally did not show decreased soil water content. The lower plant $\delta^{13}C$ in warmed plots actually suggests that the dwarf shrubs were less efficient in their water use (Ehleringer 1991), similar to the results of Welker et al. (1993), whereas higher $\delta^{13}C$ would be the expected response if the tents as a side-effect significantly reduced the plant water uptake through reduced precipitation input.

Effects of shade on plant $\delta^{13}C$

We observed a $\delta^{13}C$ decrease of 0.6–1.3‰ (*E. hermaphroditum*) or 1.2–2.2‰ (*C. tetragona*) in shaded plants in our experiment, showing that the long term C_i/C_a was higher for shaded plants than for controls. As the data on biomass, N and chlorophyll content suggest that plant growth and assimilation was not constrained by shading, the higher C_i/C_a in shaded plants was probably caused more by a relative increase in the stomatal conductance as compared to the controls than by reduced assimilation. This could e.g. be due to an altered temperature regime: a 0.6° C (heath) or 0.8° C (fellfield) lower daytime *C. tetragona* shoot temperature and inverted effects in the night (Havström et al. 1993). The decreased $\delta^{13}C$ with shading corresponds with forest ecosystem studies where lower canopy leaves had more negative $\delta^{13}C$ (i.e. higher C_i/C_a) than upper canopy leaves (Francey et al. 1985; Ehleringer et al. 1986). A higher C_i/C_a would promote photosynthesis of shade plants when leaves are exposed to higher irradiances, e.g. during sunflecks (Farquhar et al. 1989). The same could be the case for arctic dwarf shrubs exposed to increased cloudiness in a changed climate, or to shading from mountain birch which currently may be expanding (Kullman 1993).

Soil $\delta^{13}C$; the effect of source $\delta^{13}C$ on plant $\delta^{13}C$

The $\delta^{13}C$ in the soil organic matter did not differ widely from that of the plants. The ^{13}C enrichment of the upper soil layer by 1.5‰ (heath) and 1.9‰ (fellfield) (Table 3) as compared to the $\delta^{13}C$ of the dwarf shrubs (Tables 3 and 5), which constituted the main part of the aboveground biomass, is probably due to loss of ^{13}C depleted CO_2 during decomposition (Nadelhoffer and Fry 1988). Thus, the soil $\delta^{13}C$ in the upper soil layer is controlled mainly by the plant litter $\delta^{13}C$ input (Nadelhoffer and Fry 1988; Balasdent et al. 1993).

The lack of effects of shading and warming on the soil $\delta^{13}C$, despite the clear effect of these factors on plant $\delta^{13}C$, is not surprising, considering the leaf longevity of the dominant *C. tetragona* (Callaghan et al. 1989), the slow organic matter decomposition in tundras (Nadelhoffer et al. 1992) and the relatively short duration of the experiment. Hence, it is not easy to explain the small (but significant) decrease in soil $\delta^{13}C$ with fertilizer application, which was the only treatment factor that did not influence plant $\delta^{13}C$.

Feedback of C from litter and soil to the plants through ericoid mycorrhizal fungi, which colonize the roots of both species intensively (Michelsen et al. in press), is possible. A recent study by us which concerns the leaf $\delta^{15}N$ of plants from the heath and the fellfield (Michelsen et al. in press) indicates that the ericoid mycorrhizal plant species access another pool of soil N from the non- or arbuscular mycorrhizal species, and that this pool is a fraction of the organic N in the soil. This pool is most likely the major contributor of N to the ericoids, but we are presently unable to calculate the exact amount of organic N (and C) which is assimilated by ericoid mycorrhizal roots. Fractionation of C isotopes during microbial transformations of soil organic matter is also possible, which could affect the isotopic signature of the C source assimilated by the mycorrhizal roots. Hence, C uptake from the soil has been overlooked previously as an influence on plant $\delta^{13}C$.

The environmental perturbations could affect the ericoid mycorrhizal uptake of soil N-C compounds and thus influence the plant $\delta^{13}C$. For example, inhibition of mycorrhizal function is an expected response to shading (Jakobsen 1991; Michelsen and Sprent 1994), and $\delta^{15}N$ increments in shaded *C. tetragona* suggest that this treatment reduced the uptake of organic N (Michelsen A., Jonasson S., Sleep D., Schmidt I.K., Callaghan T.V., unpublished). If uptake of soil organic C also is reduced this may affect the plant $\delta^{13}C$ and we cannot, therefore, exclude that low plant $\delta^{13}C$ by shading partly is due to inhibition of mycorrhizal uptake of organic C.

The N source could also affect the plant $\delta^{13}C$ (Raven and Farquhar 1990), but mycorrhizal uptake of organic N was not considered by them. Shifts in nitrate versus ammonium utilisation with treatments are not likely as none of the dwarf shrubs species showed nitrate reductase activity at the two sites (Michelsen et al. in press).

Potentially the air carbon source might also affect the plant $\delta^{13}C$ (Farquhar et al. 1989). In our case it is unlikely that the changes in plant $\delta^{13}C$ in the shaded or the temperature treatments were caused primarily by enhanced photosynthetic assimilation of CO_2 emitted from the soil. Corresponding to the results of Chapin and Shaver (1985), the CO_2 concentrations under the shading screens and the open top greenhouses, both at the soil surface, in the canopies (where leaves of *C. tetragona* and *E. hermaphroditum* are found in the uppermost part) and above the canopies, were the same as in unperturbed plots both in the low and the high Arctic (Havström et al. 1993; Welker et al. 1993), probably because air movements in the hessian tents and the open top greenhouses only partly are restrained in these environments.

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