

CHAPTER 8

CONSEQUENCES OF MANIPULATIONS IN CARBON AND NITROGEN SUPPLY FOR CONCENTRATION OF ANTI-HERBIVORE DEFENCE COMPOUNDS IN *SALIX POLARIS*

Abstract The concentration of carbon-based anti-herbivore defence compounds is key to herbivore utilisation of forage. Production of phenolics and condensed tannins in boreal woody plants are known to reduce grazing pressure. Their production depends, among others, on the availability of nutrient resources, especially nitrogen, relative to the availability of assimilates. The carbon-nutrient balance hypothesis (Bryant *et al.* 1983) predicts a decrease in the concentration of carbon-based defence compounds with increased availability of nutrients. In a High Arctic heath, I manipulated the carbon-nitrogen balance of Polar Willow (*Salix polaris*) in a factorial, multi-level fertilising and shading experiment. Additionally, other plots were subject to elevated temperature. After two years, shading and, to a lesser extent, fertilisation had caused an increase in nitrogen concentrations of the leaves, which were highly and negatively correlated with lower phenolic and condensed tannin concentrations. Elevated temperature caused no such effects, but increased growth significantly, while shading reduced shoot biomass, and fertilisation had no detectable effect. These results are consistent with the CNB-hypothesis. It is unlikely, however, that environmental changes due to Global Climate Change will have a major impact on defence chemistry, as very high shading (> 70%) and fertilisation levels (> 1.5 g·m⁻²·y⁻¹) were needed to produce significant deviations from controls.

INTRODUCTION

The consequences of Global Climate Change are predicted to be especially pronounced in the Arctic (Intergovernmental Panel on Climate Change 1990; 1998), specifically elevated temperatures by several degrees and increases in cloudiness and precipitation. In various field experiments it has been shown that warming, increased nutrient availability and light attenuation will affect plant growth (reviewed in Dormann & Woodin 2001). Much less is known about the consequences of environmental changes on plant chemistry. In particular with respect to substances important to herbivores, such as nitrogen and grazing deterrents like phenolics and tannins, our ecological understanding is poor. Chapin *et al.* (1986a) have been able to show how nitrogen and sugar fractions of tundra plants vary across the season, and Laine & Henttonen (1987) have tried to relate interannual differences in microtine density to weather-induced differences in phenolic and nitrogen content of blueberry (*Vaccinium vitis-idaea*). Data on the effects of environmental manipulations on defence chemistry are virtually absent for the High Arctic, however (for an exception see Van der Wal *et al.* 2000c).

The anti-herbivore defence of woody arctic plants is generally carbon-based (Bryant & Kuropat 1980; Bryant *et al.* 1991b), probably because nitrogen is the limiting nutrient in these systems (Shaver & Chapin 1986) and nitrogen-based defence is thus too costly (Chapin 1989). It has been proposed (Bryant *et al.* 1983) that growth limitations due to low nutrient availability lead to a relative surplus of assimilates, which is then invested into carbon-based defence as a “carbon overflow” mechanism (for a review see Hartley & Jones 1997). Consequently, major changes in the balance of carbon and nutrient would lead to changes in the amount of carbon-based defence (Bryant *et al.* 1983).

Global climate change will influence carbon assimilation (e.g. shading due to increased cloud cover) and nutrient supply (e.g. fertilisation through increased mineralisation in warmed soils, Nadelhoffer *et al.* 1991). Consequently both shading and fertilisation should increase the availability of the limiting nutrient nitrogen relative to carbon, but decrease carbon-based secondary compounds. However, elevated air temperature may lead to an increase in carbon assimilation, as photosynthesis of arctic plants is sensitive even to small increases in air temperature (Larcher 1995), while it may also increase nutrient mineralisation, therefore providing more nitrogen to the plant. The net effect on defence chemistry will

depend on the relative magnitude of the environmental changes and the foliar chemistry responses to them.

This study investigates the consequences of the manipulation of light, nutrient availability and temperature on the concentrations of nitrogen, phenolics and condensed tannins in the leaves of *Salix polaris*. By varying shade and fertilisation at four levels in a factorial design, a whole range of different carbon-nitrogen ratios is created, enabling an estimation of the sensitivity of anti-herbivore defence in *Salix polaris* to changes in the environmental factors manipulated. The experiment also allows us to discuss various defence allocation hypotheses differing in their predictions of the effects of carbon-nitrogen imbalances.

METHODS

The study was carried out in Semmeldalen, Svalbard (77.90 °N 15.20 °E), a valley c. 20 km south of Longyearbyen, Svalbard. All experimental plots were situated in a representative patch of *Salix polaris*-heath (Rønning 1996), over an area of c. 100 by 50 m. The whole site was enclosed by an electric fence to prevent disturbance by reindeer.

Shading × fertilisation experiment

Plots of 0.5 by 1m were established in early June 1999. Treatments consisted of a full-factorial combination of four levels of fertiliser (0, 0.5, 1.5 and 5 gN m⁻² a⁻¹) and four levels of shading (0, 53, 72 and 83%). Each treatment was replicated three times, and replicates were arranged in a randomised block design. The fertiliser (ammonium nitrate, dissolved in 2 l of water per plot) was applied in rain on five occasions during the growing seasons of 1999 and 2000. Shading was attained by placing a wire-based shading frame with one, two or three layers of optically neutral greenhouse shading cloth (B&Q, U.K.) over the plot, reducing both direct and diffuse radiation into the plots.

Light availability under the shading frames was measured with a deltaT microvolt integrator fitted with a PAR photosensor (deltaT, Cambridge, U.K.). Air temperature at soil level was recorded with a RS temperature data logger (Radio Supply, U.K.), recording hourly over the whole experimental period. Soil water content was measured with a soil conductivity insertion probe (SCIP, CEH Wallingford, U.K.) on 15.6., 28.6., 1.8. and 27.8.1999, and 28.7.2000.

At the end of each season all shoots from a 25 × 25 cm square were counted and harvested from each treatment plot for determination of *Salix polaris* leaf biomass. Dead leaves were manually sorted and discarded, green leaves were counted, shoots and leaves were dried at c. 45°C for one week, transported back into the lab and re-dried before weighing. Leaves were then ground and analysed for total carbon and nitrogen using a CNS autoanalyser (Fisons, U.K.). Phenolic content of leaves were measured following the modified Prussian blue assay using tannic acid as standard and condensed tannins by the acid butanol method using purified quebracho tannin as standard (Hagerman 1998).

Elevated air temperature experiment

15 pairs of plots (60 cm diameter) were carefully selected. The experiment consisted of warmed plots and controls, randomly assigned to the paired plots. Treatment plots were subjected to warming over most of the growing season in 1999, beginning on the 16.6.1999, i.e. about ten days after snowmelt, to 29.8.1999, and over the whole snowfree period in 2000 from 14.6.2000 until harvest (1.8.2000). Greenhouses of a modified “cloche” type (Strathdee & Bale 1993) were used to raise air temperature. They consisted of an open cylinder (Ø 57 cm, height 45 cm) made of a sheet of transparent plastic, with a thin, optically neutral polyester gauze as a roof. For further details see chapter 5. Leaf material was sampled only in 2000 and treated the same way as mentioned above.

Statistical Analysis

All data were analysed with the GLM procedure of SAS (SAS Institute Inc. 1989), using block as random factor and treatments fixed effects. To separate treatment from year effects, years were analysed separately for the shading-fertilisation experiment. The interaction of shading and fertilisation was never significant at $P < 0.1$ and thus removed from the statistical model. Shoot biomass was used as a covariate to correct for concentration or dilution effects of reduced or increased growth, respectively (Koricheva 1999).

Table 1 Environmental variable responses to shading. Temperature measurements are not replicated, values in brackets are standard deviation over the experimental period, degreedays are number of days above 0°C. Soil water content (% water content) is given with standard errors in brackets (N = 12, due to pooling of fertilisation treatments). There is a significant effect of shading on soil water content ($F_{3, 218} = 3.75$, $P < 0.05$), which is also dependent on the date of measure (interaction: $F_{12, 218} = 12.18$, $P < 0.001$).

| | shade | 0% | 53% | 72% | 83% |
|--------------------|------------|-------------|-------------|-------------|-------------|
| Temperature | mean | 8.9 (5.9) | 9.1 (5.0) | 7.6 (4.5) | 7.6 (4.6) |
| | degreedays | 629 | 660 | 554 | 552 |
| Soil | 15.6.1999 | 23.3 (1.06) | 24.4 (1.47) | 26.8 (1.44) | 26.6 (0.99) |
| water | 28.6.1999 | 27.7 (1.45) | 27.5 (1.62) | 28.5 (0.79) | 27.8 (0.66) |
| content | 1.8.1999 | 18.6 (1.32) | 18.0 (1.01) | 18.7 (0.92) | 18.8 (0.59) |
| | 27.8.1999 | 19.6 (1.37) | 19.5 (1.21) | 20.3 (0.86) | 20.0 (0.84) |
| | 28.7.2000 | 20.2 (1.16) | 23.1 (0.87) | 23.7 (0.63) | 25.1 (1.37) |

RESULTS

Environmental parameters

Temperatures were altered by shading cloth (Table 1). Both 73 and 82% shading plots were c. 1.3°C cooler than ambient, while the 53% shading treatment caused slight warming. This led to a cumulative temperature difference in degreedays compared to control of +5% for the 53% shading and -12% for the 72 and 83% shading treatments. Shading treatments differed only little in soil water content on any of the four dates (Table 1). An effect of shading on soil water content was detectable, but more pronounced on wet days (Table 1). It was however lower than the variability over time. Fertilisation had no detectable effect on soil water content.

Air temperature in the greenhouses was on average 2.3°C higher than in controls (11.8 °C vs. 9.5 °C; $F_{1, 3288} = 103.2$, $P < 0.0001$). Soil water content at sampling date was significantly lower under elevated temperature (18.7 ± 2.00 % vol vs. 22.6 ± 1.78 % vol; $F_{1, 28} = 8.64$, $P < 0.01$).

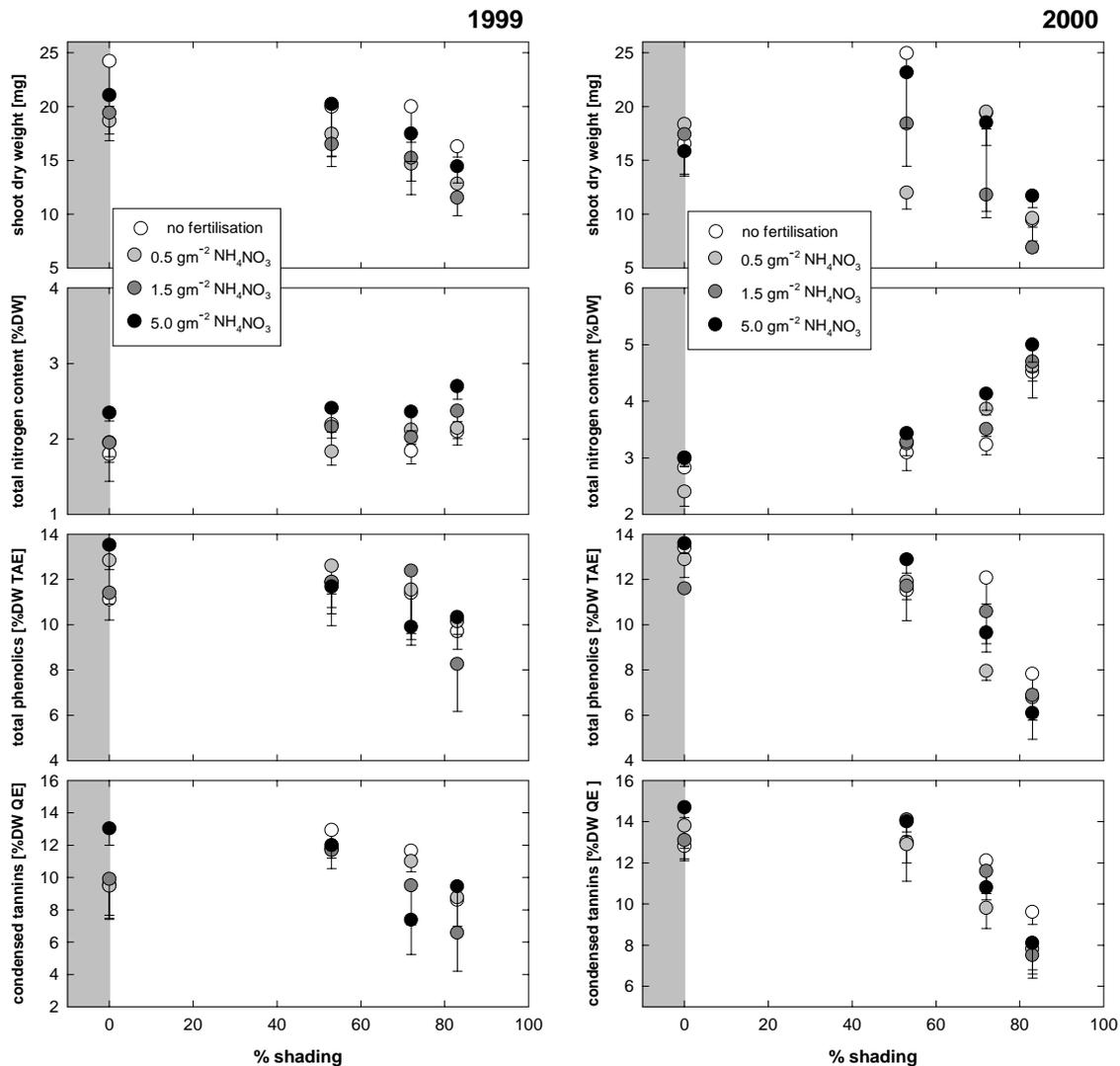


Fig. 1 Shoot biomass, leaf nitrogen, phenolic and condensed tannin content of *Salix polaris* in the four shading treatments in a) 1999 and b) 2000. Shading of points indicates level of fertilisation. Error bars indicate -1 SE.

Shading \times fertilisation experiment

Shoot biomass

Above-ground shoot biomass was significantly reduced in shaded treatments in both years (Fig. 1, Table 2). However, this decrease becomes apparent only at the highest level of shading. Fertilisation surprisingly had a transient negative effect on shoot biomass in the first season (Fig. 1a), which disappeared in the second (Fig. 1b, Table 2).

Table 2 Statistical results of treatment effects on shoot biomass, nitrogen content and phenolic and condensed tannin content of *Salix polaris*.

| factor | df | Shoot biomass | | | Nitrogen | | | Phenolics | | | Condensed tannins | | |
|------------------------|----|---------------|------|-------|----------|-------|-------|-----------|-------|-------|-------------------|-------|-------|
| | | MS | F | P | MS | F | P | MS | F | P | MS | F | P |
| 1 block | 2 | 65.96 | 5.40 | 0.009 | 1.098 | 5.40 | 0.039 | 28.56 | 4.83 | 0.040 | 43.99 | 8.41 | 0.006 |
| 9 biomass | 1 | -- | -- | -- | 0.603 | 4.23 | 0.022 | 10.66 | 2.11 | 0.136 | 4.29 | 0.80 | 0.458 |
| 9 shading | 3 | 106.46 | 8.72 | 0.000 | 1.312 | 9.21 | 0.000 | 16.75 | 3.31 | 0.031 | 5.91 | 1.10 | 0.361 |
| 9 fertilisation | 3 | 53.22 | 4.36 | 0.010 | 0.088 | 0.61 | 0.610 | 0.71 | 0.14 | 0.936 | 3.85 | 0.72 | 0.548 |
| error | 38 | 12.21 | | | 0.143 | | | 5.06 | | | 5.37 | | |
| 2 block | 2 | 133.08 | 5.16 | 0.010 | 6.240 | 21.75 | 0.004 | 76.11 | 26.62 | 0.000 | 34.12 | 12.77 | 0.006 |
| 0 biomass | 1 | -- | -- | -- | 1.233 | 8.88 | 0.001 | 5.23 | 2.10 | 0.136 | 9.23 | 5.61 | 0.007 |
| 0 shading | 3 | 235.40 | 9.14 | 0.000 | 5.240 | 37.74 | 0.000 | 56.36 | 22.65 | 0.000 | 25.20 | 15.31 | 0.000 |
| 0 fertilisation | 3 | 41.00 | 1.59 | 0.207 | 0.509 | 3.67 | 0.020 | 4.11 | 1.65 | 0.193 | 0.72 | 0.44 | 0.726 |
| error | 38 | 25.77 | | | 0.139 | | | 2.49 | | | 1.65 | | |

Nitrogen and carbon content of leaves

Nitrogen content of leaves was significantly correlated with shoot biomass, both in 1999 and in 2000 ($r = -0.305$, $P < 0.05$ and $r = -0.447$, $P < 0.001$; Pearson correlation), indicating that some leaves had a lower N-content simply because they were heavier (dilution by growth). To correct for this (Koricheva 1999), shoot weight was used as covariate in the analysis (see Table 2).

Salix polaris exhibited a significant increase in nitrogen content in response to shading in both years (Table 2). However, in 1999 this increase can only be observed at the highest level of shading, while in 2000 it is a pervasive trend, leading up to a 100% increase in leaf-N in fertilised plus shaded plots (Fig. 1). In 2000 the fertilisation treatment also caused a significant increase in leaf nitrogen content (Fig. 1b, Table 2). Leaf nitrogen levels were higher in 2000 due to differences in phenological stage at the date of sampling. This took place a week earlier, and the season began about one week later, resulting in a phenological difference between the 1999 and 2000 sampling of almost two weeks.

Leaf carbon content was less responsive than leaf nitrogen: only in the second year was shading significant ($F_{3, 39} = 4.76$, $P < 0.01$), while fertilisation was never significant. The actual difference between shaded and control plots was very small, however (control: $47.1 \pm 0.36\%$; 85% shading: $46.1 \pm 0.32\%$).

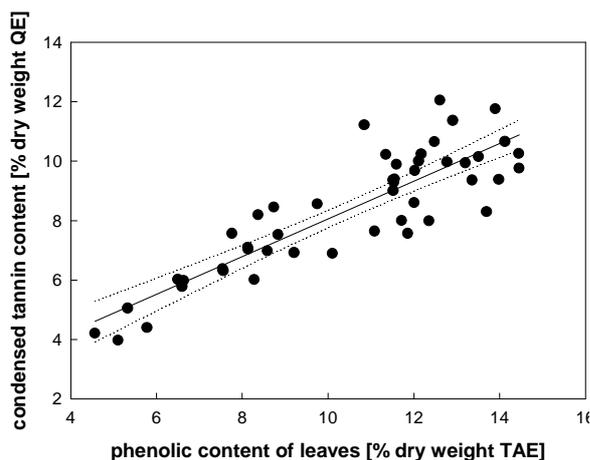


Fig. 2 Correlation plot of phenolic and condensed tannin content in 2000. Regression equation for the indicated line (and 95% confidence limits) is $y = 1.708 + 0.635 \cdot x$, $R^2 =$

Defence chemistry

Levels of anti-herbivore defence were generally high, with phenolics at c. 14 %dry weight tannic acid equivalents (TAE) and condensed tannins at c. 13 %dry weight quebracho equivalents (QE). As for nitrogen, phenolic and condensed tannin concentrations were significantly but positively correlated with shoot biomass in both years, and shoot biomass was used as covariate in the statistical analysis (Table 2).

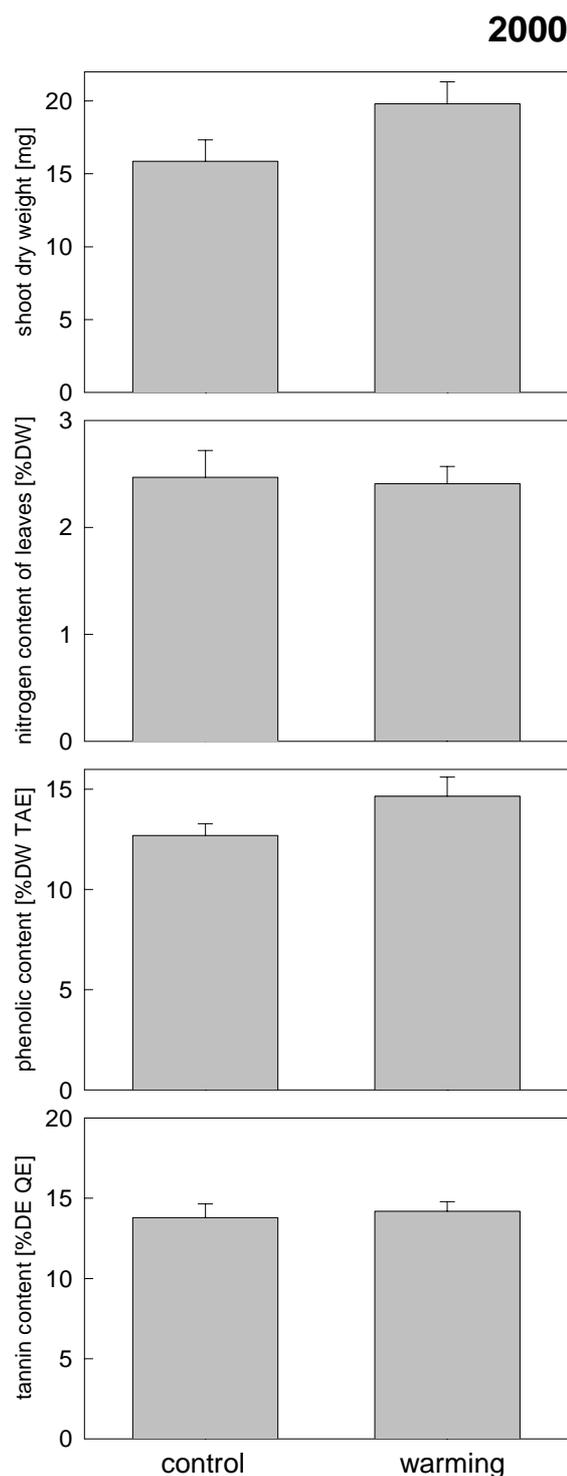


Fig. 3 Effect of elevated air temperature on shoot biomass, leaf nitrogen, phenolic and condensed tannin content of *Salix polaris*.

Phenolic concentrations showed too much treatment-independent variation in 1999 to detect significant effects of shading or fertilisation, although the highest level of shading seems to cause a decrease (Fig. 1). In 2000, this became much clearer, and the effect of shading was consistent over all treatment levels (Fig. 1; Table 2), indicating an over-proportional reduction of phenolics with increasing shading. It reduced levels of phenolics to a minimum of 50% of controls. Fertilisation had no detectable impact in either of the two years (Table 2).

The concentration of condensed tannins followed much the same pattern as phenolics (Fig. 1). However here the shading effect is significant for both years (Table 2). Again, fertilisation was without consistent effect.

The similarity between the responses of phenolics and condensed tannins is reflected in a very high correlation for 2000 ($r = 0.871$, $P < 0.001$; Fig. 2), while this correlation was not apparent in 1999 ($r = 0.149$, $P = 0.322$).

Elevated air temperature experiment

Shoots in the warmed plots grew significantly heavier than in controls ($F_{1, 14} = 5.99$, $P < 0.05$; Fig. 3). However, nitrogen, carbon, phenolic and tannin content of leaves (Fig. 3) were not significantly altered by the manipulation of air temperature ($P > 0.56$ for all comparisons). Here, no significant correlation between shoot weight and chemical properties could be detected ($P > 0.42$ for all co-variate regressions), indicating that growth was not causing a dilution of either nitrogen or defence compounds.

DISCUSSION

As predicted by the CNB-hypothesis (Bryant *et al.* 1983), carbon-based defence compounds decreased as a function of nitrogen content (Fig. 4). Manipulating shading and fertilisation increased nitrogen concentrations in *Salix polaris*, leaves. Shoot biomass explained some of the variation in defence concentrations, but nitrogen content was much more important (on average 20% of variance in defence explained by shoot weight and 60% by N content; as both factors were correlated [$r = -0.447$, $P < 0.001$, i.e. lighter shoot have higher N content] nitrogen content as covariate reduced the importance of shoot weight to a non significant level when both factors were included). The low light availability presumably led to a carbon limitation of growth, which the plants tried to compensate by a higher amount of assimilation-linked proteins (such as RubisCO) in their leaves (Bryant *et al.* 1983). It has been shown that a higher leaf nitrogen concentration is correlated with a higher photosynthesis (Field & Mooney 1986; Price *et al.* 1989; Baddeley *et al.* 1994). This explains the increased N content of the leaves with shading. As for the decrease in the concentration of defence compounds, the CNB hypothesis suggests a higher priority for allocation of newly assimilated carbon to leaf growth to overcome the carbon limitation than for defence. On the other hand, since defence concentrations of both compound groups measured here stay constant over a wide range of light conditions, *Salix polaris* appears able to buffer much of the treatment perturbation. Moreover, fertilisation could not cause any change in the defence concentrations, despite a significant effect on growth in the first and nitrogen content in the second season. This hints again at a high buffering capacity, a high priority of maintaining the high defence levels or a high level of carbon surplus.

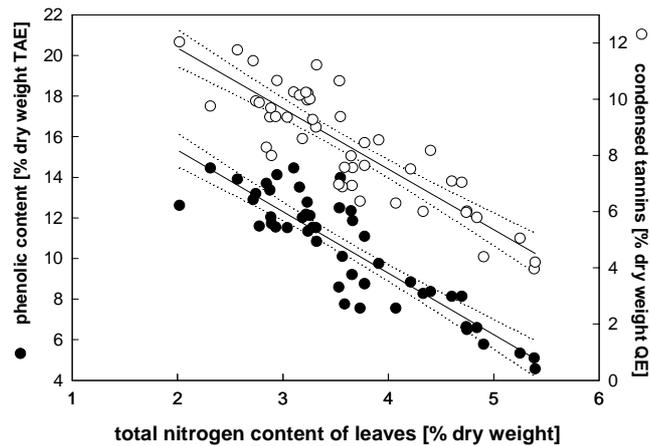


Fig. 4 Correlation of phenolic and condensed tannin content on leaf nitrogen content in 2000. Regression equation for the indicated line (and 95% confidence limits) is $y = 21.41 - 3.03 \cdot x$, $R^2 = 0.791$ for phenolics and $y = 16.12 - 2.15 \cdot x$, $R^2 = 0.749$ for condensed tannins.

A test of hypotheses

In this study I measured concentrations of two groups of carbon-based secondary compounds, both common and widespread in woody plants (Harborne 1991; Zucker 1983). Although both groups are extremely heterogeneous and consist of many different compounds, assessing their response to treatment conditions is a

useful way of understanding defence allocation. Firstly, because sometimes the decrease in one type of phenolic is compensated by the increase in another type (Hartley & Jones 1997), individual phenolic glycoside concentrations do not indicate the net effect. This is even more true for species where not all types of compounds are known. Secondly, this same compensatory mechanism might also be acting across classes of defence types (e.g. tannins, phenolics, terpenoids) and thus measurements of more than one substance class is required. Thirdly, phenolics and condensed tannins belong to the best-investigated defence compound classes, and the comparison with other studies may thus lead to further generalisations.

The high correlation between the responses of phenolics and condensed tannins suggests that the mechanism governing their production may use the same clues. This was predicted by the protein-competition-models (Jones & Hartley 1999), as condensed tannins, phenolics and many proteins share the amino acid phenylalanine as precursor.

Tests of the carbon/nutrient balance hypothesis (reviewed in Herms & Mattson 1992; Hartley & Jones 1997; Koricheva *et al.* 1998) have shown the following pattern: different ways of manipulating CNB (fertilisation, shading, elevated CO_2) lead to different consequences (e.g. hardly any effects of drought, Koricheva *et al.* 1998). Moreover, different secondary compounds also respond differently, with monoterpenes being generally unresponsive, while data on condensed tannins and phenolics often support the CNB hypothesis. Our results do

not support the conclusion that "plants of extremely low nutrient environments tend to be unresponsive", compared to fast growing species (Hartley & Jones 1997, p. 307). This could be due to an insufficient range of experimental conditions in previous experiments, which did not include the intensity leading to significant changes in our study (see Fig. 2).

With respect to the manipulation of light availability and fertilisation, most recent studies lend support to the CNB, despite its intellectually unsatisfactory lack of foundation in plant physiology (Hamilton *et al.* 2001). The increase in phenolics and condensed tannins with light intensity led Mole *et al.* (1988) to suggest an overflow mechanism for the production of carbon-based defence. For arroyo willow (*Salix lasiolepis*) fertilisation effects followed the predictions of the CNB hypothesis (Price *et al.* 1989). Both fertilisation and shading treatments caused an increase in nitrogen and decrease in phenolics and tannins in Sitka spruce (*Picea sitchensis*), but had no significant effect on nitrogen ($P = 0.07$), phenolics and lignin in heather (*Calluna vulgaris*) (Hartley *et al.* 1995; but see Iason & Hester 1993). Gebauer *et al.* (1998) report a significant decrease in phenolics and tannins with higher level of fertilisation loblolly pine (*Pinus taeda*), but provide no data on nitrogen content. Low light intensities significantly affected leaf concentrations of nitrogen, condensed tannins and phenolics in quaking aspen (*Populus tremuloides*) (Hemming & Lindroth 1999). Moreover, concentrations of condensed tannins correlated with nitrogen content, but not with that of phenolic glycosides. This study is also the only I am aware of that found synergistic effects of shading and fertilisation on any defence compound, in this case condensed tannins (Hemming & Lindroth 1999). That these changes in defence chemistry also translate into preference by herbivores is shown in the example of moose (*Alces alces*) grazing shaded and fertilised Scots pine more than controls (Edenius 1993) or Orkney vole (*Microtus arvensis orcadensis*) consuming preferably treated Sitka spruce (Hartley *et al.* 1995; see also Bryant & Kuropat 1980; Bryant *et al.* 1991b). Overall, our study provides further support for the carbon-nutrient balance hypothesis, as do most studies that manipulated light and/or nitrogen availability (but see Hamilton *et al.* 2001).

Global Climate Change and anti-herbivore defence

Our data provide a first and crude estimate for the impact of some environmental changes on plant anti-grazing chemistry. Although these experiments ran only for

two years, cautious extrapolations can be attempted. In this context the resilience of *Salix polaris* growth and leaf chemistry to fertilisation and, somewhat less, shading are remarkable: growth and N content, phenolics and condensed tannins only decreased noticeably in the extreme shade (Fig. 2). No difference in leaf chemistry could be detected in the warming experiment, despite a significant growth response. This indicates that for *Salix polaris* it is unlikely that the projected climate changes will lead to any major change in anti-herbivore compounds or N concentration. For its herbivores this probably means a slightly increased food supply due to the increased growth under warmer conditions.

In how far these conclusions are valid for other woody species remains to be seen. Moreover, the slow growth and high reliance on storage (Chapin *et al.* 1990; Grime & Campbell 1991; Iwasa 1997) of arctic plants will buffer minor climatic effects for probably some years. Consequently I can not anticipate the major implications of altered defence chemistry due to climate change for herbivore population dynamics in the High Arctic.

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