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Simulated Environmental Change Has Contrasting Effects on Defensive Compound Concentration in Three Alpine Plant Species

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Abstract

Environmental change, caused by nitrogen deposition and temperature increase, is predicted to affect allocation to carbon-based secondary compounds (CBSCs) in plants, due to changes in their internal carbon resources. The CBSCs are considered important for plant resistance to biotic and abiotic environmental stresses, such as herbivory, pathogen attacks, and UV radiation. To determine how allocation to putative defense compounds is affected by N deposition and increased temperature, we analyzed the composition of CBSCs in leaves of three arctic-alpine plant species: *Bistorta vivipara*, *Dryas octopetala*, and *Salix reticulata* after 5 years of warming (by open-top chambers) and experimental nutrient addition in an alpine *Dryas* heath in southern Norway. The dry weight of leaves increased after nutrient addition and warming combined with nutrient addition in all three species, while the weight of *D. octopetala* leaves also increased with warming alone. Individual chemical compounds or compound groups reacted to the treatments to different degrees and in different directions in the three species. The total concentration of CBSCs changed significantly only in *S. reticulata*, where it decreased in plots with nutrient addition combined with warming. Shading caused by taller vegetation in these plots might have bigger effects on the CBSC concentration than the direct changes in nutrient availability and temperature. *Dryas octopetala* had the highest concentration of CBSCs among the three species and was least affected by the treatments. Our results show that increased N availability and temperature influenced the level of carbon-based defense in some alpine plants but not others, indicating species-specific C-allocation responses to environmental change. Consequently, environmental changes may differentially affect defense abilities of alpine plant species, which could possibly contribute to future changes in interspecific competitive relationships and subsequently species composition of alpine plant communities.

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Introduction

The increase in atmospheric nitrogen (N) deposition (Galloway et al., 1995) and temperature (IPCC, 2007) is a major concern in northern ecosystems, which are typically nutrient- and temperature-limited with slow rates of growth, decomposition, and N mineralization (e.g. Körner, 1999; Bobbink et al., 1998; Chapin and Shaver, 1996). Climate change experiments in arctic and alpine environments show that most plants increase their vegetative growth and reproductive effort and success after increases in temperature (e.g. Arft et al., 1999) and/or nutrient availability (e.g. Dormann and Woodin, 2002). However, the speed and amplitude of individual plant responses often differ among species (e.g. Chapin and Shaver, 1985; Klanderud, 2008), with large consequences for the competitive relationship between species and secondarily on community composition and diversity of plant communities (Klanderud and Totland, 2005; Walker et al., 2006). The aim of the present study was to investigate if increased nutrient availability and temperature also affect allocation to putative defense compounds in arctic–alpine plants, and if responses to environmental change differ among species.

Most plants produce carbon-based secondary compounds (CBSCs) that contribute to their defense against herbivores (e.g. Tallamy and Raupp, 1991; Rosenthal and Berenbaum, 1991), pathogens (Kuc, 1982; Grayer and Harborne, 1994), other plants (Chou, 1999), and UV-B radiation (Li et al., 1993; Landry et al., 1995). In higher plants the CBSCs are phenolic compounds (including phenolic acids, flavonoids, lignins, and tannins) and terpenoids. The relatively high, but also varying, concentrations of these compounds in plants, which involve an extensive use of carbon (C) resources, have puzzled researchers for decades (see Stamp, 2003). Although CBSCs may possibly have other roles than defense they are commonly called defense compounds, and this expression will also be used in this article even though we do not test the actual roles of the compounds.

Several hypotheses have been developed to explain how carbon is allocated within the plant: the Growth-Differentiation-Balance Hypothesis (Loomis, 1953; Herms and Mattson, 1992), the Carbon Nutrient Balance Hypothesis (Bryant et al., 1983), and the Resource-Availability Hypothesis (Coley et al., 1985; Bazzaz et al., 1987). These hypotheses generally assume that the synthesis of CBSCs is limited by the availability of photosynthates and that growth processes dominate over differentiation and/or production of CBSCs when growth conditions are favorable. However, if growth is limited by nutrients, allocation towards defense will increase. These hypotheses have been widely discussed and tested

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(Hamilton et al., 2001; Koricheva et al., 1998; Stamp, 2003), but none of them seems to give a clear picture (e.g. Treutter, 2006).

It is evident that the level of defense must be influenced also by genetic and environmental factors, and the interaction between them. Genetically based defense is commonly denoted constitutive, consisting of innate compounds that are synthesized during the normal development of plant tissue, while environmentally regulated defense is referred to as inducible, and synthesized by plants in response to physical injury, infection, or stress (Treutter, 2006). The relative importance of environmental and genetic factors varies considerably among species (Hamilton et al., 2001). The relationship between resource status and constitutive/inducible defense is unclear and little tested. It is, however, predicted that some defense must be prioritized before growth even in low resource situations, but that the prioritization between growth or defense may vary among species (e.g. Tuomi et al., 1991; Stamp, 2003). In general, inherently fast growing species have lower defense than slow-growing ones (Bryant et al., 1983; Coley et al., 1985). Furthermore, evergreens are expected to invest more in leaf defense than deciduous species (Tuomi et al., 1991) because of the importance to protect the longer living leaves, but also because evergreens store their carbon resources in leaves while deciduous species have their main storage in stems and roots (Tuomi et al., 1988).

Both temperature and N availability may influence the C-status of a plant, and thus also affect levels of plant defense. Increased availability of N generally increases growth, and thus also the use of C, and the expected result is a reduced allocation to CBSCs (Bryant et al., 1983). This is generally confirmed for non-terpenoid compounds (Koricheva et al., 1998; Hamilton et al., 2001). Increased temperature should provide more C available for synthesis of CBSCs, as photosynthesis, and thus C harvesting, increases with temperature up to a threshold for most plants (e.g. Körner, 1999). For example, the photosynthesis (PPFD) of *Dryas octopetala* significantly increased under a 3.5°C temperature elevation treatment by open-topped polyethylene tents at Svalbard (European High Arctic), and the C gain was ca. 10% higher than at ambient temperature (Wookey et al., 1995). Results from experiments investigating the effects of temperature increase on CBSCs are contradicting, and in a meta-analysis, Zvereva and Kozlov (2006) showed that elevated temperature generally increased phenolic compounds in the green parts of gymnosperms but decreased concentrations in angiosperms. The majority of fertilizer and temperature enhancement experiments outdoors analyzing CBSCs have been on tree species (Koricheva et al., 1998; Zvereva and Kozlov, 2006), and the effect of environmental changes on plant defense in N and temperature limited arctic and alpine plant communities have been little investigated (but see Dormann, 2003; and Hansen et al., 2006).

We tested the effect of a five-year warming and nutrient addition experiment on the level of CBSCs (HPLC-phenolics and condensed tannins) in three arctic-alpine plant species (*Bistorta vivipara*, *Dryas octopetala*, *Salix reticulata*) using a factorial block experiment in an alpine plant community in southern Norway. Based on resource availability hypotheses, we expected a reduced concentration of CBSCs after nutrient addition, while increased temperature could either increase CBSCs due to increased photosynthesis, decrease due to a greater mobilization of nutrients, or cause no response if these two effects cancelled each other. Finally, we hypothesized that the effect of environmental changes on CBSCs may be stronger in the herb *B. vivipara* and the deciduous dwarf shrub *S. reticulata* than in the wintergreen dwarf shrub *D. octopetala*, because we (ref. Tuomi et al., 1991; Stamp, 2003) expect plants with annual leaves to have a larger pool of genetically decided (constitutive) defense than those with perennial leaves, such as *D. octopetala*.

Material and Methods

PLANT MATERIAL AND TREATMENTS

This study was conducted on a southwest-exposed slope of a *Dryas octopetala* heath at ca. 1500 m elevation on Sandalsnuten, Finse, northern part of Hardangervidda (ca. 60°N, 7°E) in the alpine region of southwestern Norway. Mean monthly temperature during June, July, and August at 1200 m elevation at Finse is 6.3 °C (Aune, 1993), and mean monthly precipitation during the same months is 89 mm (Førland, 1993). To examine if environmental changes may affect concentration of CBSCs in plants, we collected leaf samples from a five-year-old experiment with forty 1 × 1 m plots in a randomized block design with 10 replicates (blocks). The treatments were: temperature increase (T), nutrient addition (N), temperature increase and nutrient addition (TN), and controls (C, no treatment). Open-top chambers (OTCs, see e.g. Marion et al., 1997; Hollister and Webber, 2000) increased the mean temperature ca. 5 cm above ground by ca. 1.5°C, and soil temperature ca. 5 cm below ground by ca. 1°C. Temperatures were logged hourly during the whole growth season. Slow-released granular NPK (ca. 10 g N, 2 g P, and 8 g K per m^2/growing season) fertilizer increased nutrient availability. These amounts are in line with other climate change experiments (e.g. Chapin et al., 1995; Press et al., 1998; Shaver and Jonasson, 1999). See Klanderud and Totland (2005) for details on experimental set-up.

We used three species growing naturally in the experimental plots to test our hypotheses. These species represent different functional groups that may have differential chemical defense strategies (Tuomi et al., 1991; Stamp, 2003). *Dryas octopetala* (Rosaceae) is a wintergreen dwarf-shrub growing in dry habitats where snow melts early, on gravel and rocky barrens, and often forms distinct heath communities on calcareous soils in arctic-alpine environments. *Salix reticulata* (Salicaceae) is a deciduous dwarf-shrub, forming mats in moist tundra on gravel and sand beaches, stream banks, colluvial slopes, edges of frost polygons, and snowbeds, usually in places well protected by winter snow cover, often but not exclusively on calcareous substrates. *Bistorta vivipara* (Polygonaceae) is a perennial herb with a short, thick stem and a few spread and oblong leaves. Green/black/red bulbils are produced below the tiny flowers. In the arctic-alpine it mostly occurs on meadows and heaths, generally on nutrient rich substrates. Mats with *D. octopetala* and *S. reticulata* in mixture generally dominated the experimental plots, but *S. reticulata* was absent from two blocks. *Bistorta vivipara* plants occurred individually within the mats.

We sampled leaves randomly from the center of each plot in early August 2004. For *D. octopetala* and *S. reticulata* we sampled three fully developed leaves, from three different ramets from each plot, while the lower abundance of *B. vivipara* plants allowed us to collect only one mature leaf from each plot. The leaves were put into small plastic bags with silica gel, and stored for one week at room temperature for drying, and then transferred to a refrigerator (4°C) for one week, and finally placed in the freezer (−18°C) and kept there until extraction (spring 2005). This drying method had been tested for the studied species in a pilot experiment, and no changes in CBSC composition and concentration were detected after one week at room temperature and one week in the refrigerator. Drying in silica gel has also been tested on *Salix purpurea* by Julkunen-Tiitto and Sorsa (2001), who

recommended it as a good method for drying/storing field-collected leaves.

EXTRACTION AND ANALYSIS OF PHENOLIC COMPOUNDS

The silica bags were removed from the refrigerator and kept at room temperature overnight. We took the dried leaf samples out from the silica bags, measured their dry weight (DW) and then removed the middle veins and stems with a scalpel. The leaf material was then transferred to pre-weighed Eppendorf vials containing one conic stainless steel bead of 5 mm diameter. We crushed the leaves to powder for 2 min in a Retsch mixer mill (Model MM301) at frequency 30.0 and then weighed the sample. After addition of 600 µL methanol (MeOH) (or 500 µL MeOH and 100 µL naringenin [internal standard] in every second sample) and mixing with an Ultra-Turrax homogenizer for 30 sec, we placed the sample in an ice bath for 15 min, homogenized it for 15 sec, centrifuged it at 15,000 rpm for 3 min and then poured the supernatant into a clean glass tube. The residue was added 600 µL MeOH, homogenized for 15 sec and again centrifuged. The last procedure was repeated twice, and the residue was then totally colorless. The supernatants were then combined and the MeOH evaporated with gaseous nitrogen. The dried extracts were stored at -18°C until analysis.

The extracts were dissolved in 300 µL MeOH, added 300 µL Milli-Q water, and analyzed on HPLC as described in Julkunen-Tiitto et al. (1996). We identified the compounds according to retention times and UV-spectra, quantified them at 270 nm, and calculated the concentrations using the following commercial standards (supplier in parenthesis): caffeic acid (Aldrich, Steinheim, Germany), chlorogenic acid (Aldrich), 4-hydroxycinnamic acid (Aldrich), salidroside (Thieme, Germany), eriodictyol-7-glucoside (Roth, Karlsruhe, Germany), picein (Extrasynthese, Genay, France), triandrin (supplied by Beat Meier, ETH, Switzerland), (+) catechin (Aldrich), myricetin-3-rhamnoside (Apin Chemicals, Abingdon, U.K.), quercetin-3-glucoside (Extrasynthese), apigenin-7-glucoside (Roth), and luteolin-7-glucoside (Extrasynthese).

Soluble condensed tannins were analyzed from the HPLC sample and insoluble condensed tannins from the dried extract residue by the acid butanol assay, as reported in Porter et al. (1986). Concentrations were calculated according to standards of purified tannin from *Betula nana* (dwarf birch) leaves.

As compounds within the same chemical group generally responded similarly to the treatments in the three studied species (Table 1), we chose to present concentrations (mg g^{-1} DW) and statistics for compound groups, and not for individual compounds when appropriate (Table 1). The individual compounds identified and the grouping are shown in Table 2.

STATISTICAL ANALYSIS

We conducted a mixed-effects ANOVA, using the GLM-module in SYSTAT 10 (SPSS Inc., Chicago, U.S.A.), with treatment as the fixed factor (with the levels control, temperature increase, nutrient addition, temperature increased combined with nutrient addition) and block as the random factor in a randomized block design to assess the treatment effect on the concentration of compounds/compound groups. After a significant treatment factor we used the Tukey HSD post-hoc test to examine which levels of the treatment factor were significantly different from each other.

Results

LEAF DRY WEIGHTS

Leaves of all three species had significantly higher dry weights (DW) in nutrient addition (N) plots than in control plots (C) (Table 1). When nutrients were added in combination with elevated temperature (TN), the increase was even higher (66, 79, and 31% for *S. reticulata*, *B. vivipara*, and *D. octopetala*, respectively). In *D. octopetala*, temperature alone (T) also increased leaf dry weight, whereas leaf weight of the two other species did not respond to this treatment (Table 1).

COMPOSITION OF CARBON-BASED SECONDARY COMPOUNDS IN THE SPECIES

In total, the identified carbon-based secondary compounds (CBSCs) constituted up to 24.0, 24.5, and 31.1% of the DW of *S. reticulata*, *B. vivipara*, and *D. octopetala*, respectively. All three species contained comparable amounts of MeOH-soluble condensed tannins (SolCT) (up to 14% of the DW in *S. reticulata* and *B. vivipara*, and 15.5% in *D. octopetala*), while *B. vivipara* contained higher amounts of MeOH-insoluble condensed tannins (InsolCTs) compared to the two other species (6.2% of the DW compared with 3.1% in *D. octopetala* and 3.2% in *S. reticulata*). The rest of the CBSCs are low molecular weight compounds (e.g. phenolic acids and flavonoids; see Table 2 for individual compounds identified), of which *D. octopetala* contained much more (12.9%) than *B. vivipara* (5.1%) and *S. reticulata* (7.4%).

EFFECTS OF TREATMENTS ON CARBON-BASED SECONDARY COMPOUNDS

The simulated environmental change had the strongest effects on *S. reticulata* secondary chemistry, where the total concentration of CBSCs was significantly reduced when both temperature and nutrient availability increased (TN; Table 1). The TN treatment reduced the concentration of all detected flavonoids compared with controls, although not significantly for luteolin-glycosides and flavan-3-ols. The MeOH-soluble condensed tannins (SolCTs) decreased in N and TN plots and increased in T plots, but only the differences between the T and N, and T and TN plots were significant (Table 1).

In *B. vivipara*, total concentration of both low molecular weight CBSCs and SolCTs tended to be reduced by the T, N, and TN treatments, but the effects were not significant. There were, however, significant effects on some of the individual compounds/compound groups. In *B. vivipara*, nutrient addition alone (N) reduced the phenolic acids, while temperature increase (T) had the opposite effect (Table 1) (only the difference between T and N was significant). Elevated temperature reduced the quercetin-glycosides, while other flavonoids (flavan-3-ols, flavonols: myricetin-glycosides and flavones: luteolin-glycosides) were not significantly affected. Nutrient addition (N) increased the MeOH-insoluble condensed tannins (InsolCTs) (Table 1).

In *D. octopetala* there was no significant effect of any of the treatments on the total concentration of CBSCs. However, salidroside concentration was significantly lower in N plots than in controls (Table 1), and all detected flavonols (the myricetin derivative and the quercetin-glycosides) were significantly reduced by the combined TN treatment. No other individual compounds or compound groups changed.

There were no significant effects of block on any of the compounds/compound groups in any of the three species.

TABLE 1

Dry weight (DW) of leaves (mg) and concentrations of carbon-based secondary compounds (CBSCs) (mg g^{-1} DW; means ± S.E.) in *Bistorta vivipara*, *Dryas octopetala*, and *Salix reticulata* at Finse. The treatments were: control (C), temperature increase (T), nutrient addition (N), and temperature increase and nutrient addition combined (TN). $n = 10$. From each plot one leaf of *B. vivipara* and three leaves of *D. octopetala* and *S. reticulata* were analysed. F- and *P*-values presented are treatment effects from a one-factor ANOVA. Results from the Tukey HSD post-hoc test are shown with lower case letters; different letters indicate significant difference ($P < 0.05$). SolCT = methanol soluble condensed tannins, InsolCT = methanol insoluble condensed tannins.

	C	T	N	TN	F	*P*
S. reticulata						
DW per leaf	5.6 ± 0.5[a]	6.1 ± 0.5[a]	9.7 ± 0.6[b]	9.2 ± 0.8[b]	9.29	<0.001
Picein	0.7 ± 0.1	1.3 ± 0.4	0.4 ± 0.0	0.4 ± 0.0	2.7	0.070
Triandrin	1.5 ± 0.1	1.7 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	0.4	0.745
Phenolic acids	9.3 ± 0.9	10.6 ± 0.6	8.1 ± 0.9	8.1 ± 0.8	1.62	0.215
Flavan -3-ol	13.4 ± 0.8	13.2 ± 1.2	12.12 ± 1.5	11.7 ± 0.9	0.595	0.625
Luteolin-glycosides	34.1 ± 2.1	32.4 ± 2.3	37.2 ± 8.0	23.6 ± 2.0	1.78	0.182
Apigenin-glycosides	4.8 ± 0.2[a]	4.3 ± 0.3[ab]	5.1 ± 0.4[a]	3.5 ± 0.3[b]	4.17	0.018
Methylluteolin -7-glucoside	10.2 ± 0.6[a]	8.6 ± 0.98[a]	8.7 ± 0.7[a]	5.7 ± 0.4[b]	7.19	0.002
Sum, low-molecular compounds	74.1 ± 3.9[a]	72.0 ± 3.3[a]	73.4 ± 8.2[a]	54.7 ± 3.9[b]	3.52	0.027
SolCT	120.3 ± 8.1[ab]	139.1 ± 5.0[a]	102.7 ± 7.0[b]	101.0 ± 5.8[b]	4.56	0.013
InsolCT	29.5 ± 1.5	28.4 ± 1.2	31.6 ± 1.3	29.7 ± 1.2	0.47	0.704
Sum all	**224.0 ± 9.6[a]**	**240.2 ± 8.6[a]**	**206.9 ± 14.3[ab]**	**185.5 ± 11.7[b]**	**3.94**	**0.022**
B. vivipara						
DW per leaf	7.1 ± 0.5[a]	7.7 ± 0.6[a]	12.1 ± 1.1[b]	12.7 ± 0.7[b]	15.50	<0.001
Flavan-3-ols	1.6 ± 0.2	1.3 ± 0.3	1.5 ± 0.2	1.9 ± 0.4	0.57	0.639
Phenolic acids	13.5 ± 0.8[ab]	16.4 ± 1.5[a]	10.6 ± 0.8[b]	14.3 ± 0.8[ab]	5.89	0.003
Myricetin-glycosides	10.2 ± 2.4	6.5 ± 1.2	8.3 ± 1.6	5.5 ± 1.0	1.78	0.176
Quercetin-glycosides	24.3 ± 1.3[a]	17.4 ± 2.9[b]	21.1 ± 1.1[ab]	18.6 ± 1.3[ab]	2.65	0.070
Luteolin-glycosides	0.7 ± 0.3	0.4 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0.56	0.647
Sum, low-molecular compounds	50.9 ± 3.6	45.8 ± 3.6	42.9 ± 2.0	42.4 ± 2.5	1.99	0.134
SolCT	142.6 ± 11.8	144.6 ± 11.2	113.5 ± 6.8	126.1 ± 8.00	1.45	0.251
InsolCT	52.2 ± 1.5[ab]	49.2 ± 2.4[a]	62.0 ± 1.5[b]	55.7 ± 1.9[ab]	0.77	0.019
Sum all	**245.0 ± 14.9**	**234.9 ± 12.3**	**218.4 ± 7.7**	**224.2 ± 6.6**	**1.33**	**0.281**
D. octopetala						
DW per leaf	5.6 ± 0.4[a]	7.3 ± 0.3[b]	6.9 ± 0.3[b]	7.4 ± 0.4[b]	6.36	0.001
Salidroside	1.4 ± 0.1[a]	1.3 ± 0.2[ab]	0.7 ± 0.1[b]	1.3 ± 0.2[ab]	6.30	0.002
Flavan-3-ols	85.7 ± 5.2	88.9 ± 4.7	77.6 ± 3.9	84.3 ± 4.8	1.03	0.394
Eriodictyol-7-glucoside	14.1 ± 0.8	12.5 ± 1.4	13.9 ± 1.3	13.0 ± 1.4	0.44	0.727
Myricetin derivative	8.6 ± 0.5[a]	9.7 ± 0.6[a]	7.9 ± 0.4[ab]	6.5 ± 0.7[b]	5.99	0.003
Quercetin-glycosides	19.6 ± 1.2[a]	16.4 ± 1.3[ab]	19.6 ± 1.1[a]	14.9 ± 1.2[b]	4.93	0.007
Sum, low-molecular compounds	129.3 ± 6.1	128.8 ± 6.9	119.6 ± 3.8	120.0 ± 6.4	0.81	0.496
SolCT	155.2 ± 10.7	151.4 ± 9.2	144.3 ± 8.5	143.1 ± 8.2	0.36	0.786
InsolCT	26.7 ± 2.3	28.0 ± 2.6	31.4 ± 2.4	29.3 ± 1.7	0.76	0.529
Sum all	**311.1 ± 9.6**	**308.2 ± 12.2**	**295.3 ± 11.6**	**292.3 ± 13.5**	**0.30**	**0.827**

Discussion

Concentrations of carbon-based secondary compounds (CBSCs) are important for plant resistance to biotic and abiotic environmental stresses, such as herbivory, pathogen-attacks, and UV-radiation (e.g. Treutter, 2006). Our results show that the effect of warming and increased nutrient availability on the concentration of CBSCs may vary both among species and among different groups of CBSCs within the same species.

In *Salix reticulata* the total production of CBSCs was significantly reduced when nutrient addition was combined with temperature increase (TN plots). The same tendency also occurred when only nutrients (N) were added, although the effect was not statistically significant. According to resource allocation hypotheses (Loomis, 1953; Herms and Mattson, 1992; Bryant et al., 1983; Coley et al., 1985; Bazzaz et al., 1987), a decrease in CBSCs is expected when nutrients are added, due to increased use of C for growth. Increased temperature, on the other hand, was expected to increase CBSCs. This is because increased photosynthesis, and thus increased C-availability, has been shown to increase with elevated temperature for arctic-alpine plants (e.g. Wookey et al., 1995). There was a slight, although not significant, positive effect of temperature on CBSCs in our T plots. In the TN plots, on the other hand, any positive temperature effect on photosynthesis in the low-stature *S. reticulata* might have been overridden by a strong shading effect by the taller grasses and forbs (Klanderud and Totland, 2005). In both the N and TN plots, the biomass of grasses and forbs was significantly higher than in the C and T plots (Klanderud and Totland, 2005). Consequently, photosynthetically active radiation (PAR) was 52 and 76% lower at ground level in the N and TN plots, respectively, compared to the control plots, whereas the PAR reduction was only 12% in the T plots (Klanderud and Totland, 2005). The dry weight (DW) of *S. reticulata*, however, increased in the N and TN plots, and therefore does not appear to be affected by shading. The decrease in CBSCs in the TN plots indicates that synthesis of secondary compounds in *S. reticulata* may be limited by C-resources, but only when photosynthesis is strongly reduced. However, it is also possible that plants actively reduced synthesis of defense compounds when the solar irradiation, and hence UV-B amounts, are reduced, independently of the resource availability. Our findings are in agreement with the results of Hansen et al.

TABLE 2

Identified low molecular phenolic compounds in the three studied species, divided into compound groups.

Compound group			Individual compounds		
			Bistorta vivipara	*Dryas octopetala*	*Salix reticulata*
		Phenolic acids	Caffeic acid Chlorogenic acid		Chlorogenic acid 3 unidentified hydroxycinnamic acids
		Other compounds		Salidroside Eriodictyol-7-glucoside	Picein Triandrin
Flavonoids	Flavan-3-ols		(+)-catechin	(+)-catechin 4 catechin derivatives	(+)-catechin
	Flavonols	Myricetin glycosides	Myricetrin 2 unidentified		
		Quercetin glycosides	Hyperin 1 unidentified	Hyperin quercetin-3-arabinoside 1 unidentified	
	Flavones	Luteolin glycosides	1 unidentified		Luteolin-7-glucoside luteolin-5-glucoside 1 unidentified
		Apigenin glycosides			Apigenin-7-glucoside
		Methylluteolin glycosides			Methylluteolin-7 glucoside

(2006), who found a significant decrease in condensed tannins in *S. herbacea* × *S. polaris* in plots with shading and shading combined with nutrient addition, while nutrient addition alone did not cause any significant effect on the concentration of condensed tannins in their experiment.

None of the treatments had any significant effect on total CBSCs or total concentration of low molecular compounds in *B. vivipara* and *D. octopetala*. However, the concentration of condensed tannins tended to decrease after N addition in *B. vivipara*. *Dryas octopetala* leaves had the highest concentration of CBSCs (ca. 31% of the DW in controls compared with 22 and 25% in *S. reticulata* and *B. vivipara*, respectively) and seems to be able to compensate for the relatively low growth response (31% higher DW in TN-leaves than in controls, compared with 66 and 73% for *S. reticulata* and *B. vivipara*, respectively) by keeping the concentration of total CBSCs close to control levels. This suggests a high priority of defense, which could be expected in slow growing species that maintain their leaves for more than one growing season (Tuomi et al., 1988, 1991). *Dryas octopetala* was the only one of the three studied species that increased leaf weight in response to increased temperature (T). This agrees with results from *D. octopetala* studies in the High Arctic Svalbard, where photosynthesis also increased significantly after three years of warming (Wookey et al., 1995), and suggests that growth of *D. octopetala* is not only nutrient-, but also carbon-limited in arctic and alpine habitats. However, the dry-weight increase may also partly be a result of increased storage of C (carbohydrates) in the evergreen leaves, not only tissue growth.

Individual compounds changed in different directions in the three study species. Variation in responses among compound groups to simulated environmental change appears to be common (e.g. Kainulainen et al., 1996; Keinänen et al., 1999; Hansen et al., 2006; Witzell and Shevtsova, 2004). Since all compounds analyzed in this study are synthesized along the same metabolic pathway (the phenylpropanoid pathway) and share the common precursor phenylalanine, the variability of individual responses cannot be explained by the treatments. Differences in resource availability should not directly affect the distribution of carbon to different CBSCs at these lower hierarchical levels (Koricheva et al., 1998).While the total level of CBSCs, according to the resource hypotheses, can be influenced by amounts of available carbon not used for growth, the proportional allocation to individual compounds depends on the specific evolutionary responses of plants to environmental stresses, such as herbivory (Tuomi et al., 1988), pathogens, UV radiation, and ozone (Koricheva et al., 1998). However, most of the significant changes found in our study are decreases due to the N or TN treatments in compounds rather far downstream the phenylpropanoid pathway, such as flavonols, flavones, and condensed tannins, while their precursors (phenolic acids and flavan-3-ols) stay more or less unchanged (although phenolic acids in *B. vivipara* are an exception) (Table 1). Condensed tannins (Collingborn et al., 2000; Heiska et al., 2008), flavonols (Mallikarjuna et al., 2004), and flavones (Sosa et al., 2004) have all been shown to have protective functions, and there are no indications in the literature that the simpler compounds should be more important in this context. It may be that in a low C resource-situation, it is beneficial to prioritize low molecular weight compounds with multiple precursor functions, which can be used to build defense in response to a specific stress (induced response) rather than complex compounds that are not easily re-metabolized.

In conclusion, we show that environmental changes may affect leaf chemistry in alpine plants, but that the magnitude and direction of change vary among species. Consequently, environmental change may differentially affect defense abilities of alpine plant species, which could possibly alter interspecific competitive

relationships and subsequently plant community composition. Our results also indicate that the indirect effect of environmental change, such as shading due to increased biomass of other species, may be more important for the CBSC status of individual species than the direct effects (increased growth). We also show that the different compound groups are differentially affected by changes in resource availability. The woody wintergreen *D. octopetala* is, as expected, least affected by the environmental changes and probably has a high constitutive defense. *Salix reticulata* and *B. vivipara*, on the other hand, which have annual leaves, seem to be more susceptible to change. The basis of the resource allocation hypotheses is that the concentration of CBSCs is controlled by the availability of C and N resources. Our results suggest resource availability is likely important in determining the level of defense in alpine plants, but it cannot be ruled out that plants adjust to the defense level needed, independently of a C-surplus. To evaluate how CBSC synthesis is prioritized in situations with variable amounts of C available in the plant, we need studies that measure carbohydrate concentration and the total C status in addition to concentrations of defense compounds.

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