Examensarbeten **2010:02**
Institutionen för skogens ekologi och skötsel

Variation in Protein Precipitation and Phenolic Content Within and Among Species Across an Elevational Gradient in Subarctic Sweden

Variation i proteinbindningskapacitet och fenolhalt inom och mellan arter över en höjdgradient i subarktiska Sverige

Foto: Björn Olofsson

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Examinator: Hjalmar Laudon, SLU, Inst för skogens ekologi och skötsel Umeå 2010

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I denna rapport redovisas ett examensarbete utfört vid Institutionen för skogens ekologi och skötsel, Skogsvetenskapliga fakulteten, SLU. Arbetet har handletts och granskats av handledaren, och godkänts av examinator. För rapportens slutliga innehåll är dock författaren ensam ansvarig.

This report presents an MSc/BSc thesis at the Department of Forest Ecology and Management, Faculty of Forest Sciences, SLU. The work has been supervised and reviewed by the supervisor, and been approved by the examinator. However, the author is the sole responsible for the content.

Abstract

This project investigated how elevation and vegetation type influences variation in plant litter phenolic content and protein precipitation capacity among and within common plant species for two different vegetation types, heath and meadow, in a subarctic ecosystem in the Abisko region of northern Sweden. As nutrient availability generally decreases with increasing elevation as a result of decreasing temperature, I hypothesised that phenolic content would increase with elevation and be higher on the heath than the meadow. To test this, the total phenolic content and protein precipitation capacity was estimated in leaf litter from 13 species in both heath and meadow vegetation across an elevational gradient ranging from 500 to 1000 meters above sea level (m.a.s.l.) in the study region. The results showed that elevation and vegetation type both had a strong impact on both variables. Total phenolic concentrations decreased with elevation for the meadow, and were greater for the heath than the meadow. Moreover, there was a general trend of decline in protein precipitation with increasing elevation for both vegetation types. Further, species that dominated at higher elevations produced litter with lower phenolic concentrations and protein precipitation capacity than did those species that dominated at lower elevations. My results are inconsistent with my hypothesis as well as with previous studies that have suggested a negative relationship between phenolic content and nutrient availability. They also highlight the need for an improved understanding of what factors drive phenolic production in plant litter, both within subarctic ecosystems and more generally.

Keywords: phenolic compounds; protein complexation; elevational gradient; subartic; fertility gradient

Sammanfattning

I det här projektet undersöktes hur fenolhalt och proteinbindningskapacitet i vanligt förekommande fjällväxter påverkas av höjd över havet och vegetationstyp (hed och äng) i ett subarktiskt ekosystem i Abisko, norra Sverige. Min hypotes vid projektets början var att fenolhalten skulle öka med höjden och vara högre i växter från hed än från äng, p.g.a. att näringsförhållandena generellt minskar med höjd över havet som ett resultat av minskande temperatur. För att studera detta mättes den totala fenolhalten och proteinbindningskapaciteten i blad från 13 arter hemmahörande i hed- och ängsvegetation över en höjdgradient som sträcker sig från 500 till 1000 meter över havet. Resultaten visade att både höjd över havet och vegetationstyp hade stor påverkan på båda variablerna. Total fenolhalt minskade med stigande höjd över havet i ängsvegetationen och var generellt högre på hed än på äng. Därtill fanns en generellt avtagande trend i proteinbindningskapacitet med höjd i båda vegetationstyperna. Arter som dominerade högre höjder innehöll lägre fenolhalter och sämre proteinbindningskapacitet än arter från lägre höjder. Mina resultat överensstämmer varken med min hypotes eller tidigare studier som har föreslagit ett negativt samband mellan fenolhalt och näringstillgång. Resultaten pekar också på behovet av en förbättrad förståelse för de faktorer som styr fenolproduktion i växter, både inom subarktiska ekosystem och mer generellt.

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Introduction

Phenolic compounds are carbon-based secondary metabolites that all terrestrial plant species are potentially capable of producing (Hättenschwiler & Vitousek 2000; Hümmer & Schreier 2008). They can be divided into two broad groups: astringent phenolics (tannins) and non-astringent phenolics (Meier & Bowman 2008). Their role in plants was long interpreted as deterring herbivores but more recent research has suggested the importance of phenols in plant-litter-soil interactions (Spencer et al. 1988; Northup et al. 1995b; Hättenschwiler et al. 2003; Eskelinen et al. 2009). Through the capacity that astringent phenolics (tannins) have to form recalcitrant phenol-protein complexes, they can play an important role in determining soil nutrient conditions (Kuiters 1990; Schimel et al. 1998; Northup et al. 1995b; Fierer et al. 2001; Bowman et al. 2004). As such, litter rich in astringent phenolics effectively inhibits decomposition through reducing the availability of essential nutrients (notably nitrogen) for plants and microbes (Baldwin et al. 1983; Hernes & Hedges 2000; Ushio et al. 2009). This leads to accumulation of organic nitrogen (N) pools (Kraus et al. 2003) which only some species can utilize, notably those with ericoid- and ectomycorrhizal fungi (Bending & Read 1996a, b). Certain plant species that produce compounds that are able to form phenol-protein complexes are also able to utilize N within these complexes, giving them a competitive advantage over coexisting species that are unable to utilize this N source (Horner et al. 1988). Further, Northup et al. (1998) suggested that plant species that produce phenolics with the capacity to form protein-phenolic complexes lead to reduced mobility of N, and therefore reduce the loss of N through leaching. Polyphenols can thus act to conserve N in nutrient-limited environments.

Many factors that affect resource availability change as a result of temperature variability associated with elevation, including characteristics of the soil microbial community, and therefore nutrient cycling and nutrient supply rates from the soil (Ruess et al. 1999). Elevational gradients are therefore powerful tools to evaluate ecological and evolutionary adaptations to temperature and associated climatic variables whenever other abiotic factors do not co-vary with elevation (Fukami & Wardle 2005; Körner 2007). It is also well known that aboveground properties such as plant species richness, productivity and standing biomass change predictably with increasing elevation (Körner et al. 1989; Körner 2003). As temperature decreases with elevation and nutrient cycling processes are slowed down, the role of phenolics in regulating nutrient availability may be expected to become increasingly important with increasing elevation.

In addition to differences originating from elevation, vegetation type is likely to affect the phenolic properties of arctic species. Subarctic ecosystems are characterised by low temperatures and short growing seasons (e.g. Björk et al. 2007) and are generally recognized as being N limited (Körner 2003; Soudzilovskaia et al. 2005). Swedish tundra is a mosaic of two vegetation types, heath (dominated by ericaceous dwarf shrubs and *Betula nana*) and meadow (dominated by herbs, sedges and graminoids), that occur at all elevations. Relative to the heath communities, the meadows are generally moister and demonstrate greater nutrient availability and more rapid cycling of nutrients (Körner 2003). In contrast to meadow communities, heath communities occur on nutrient poor patches, and are composed of species with more conservative growth strategies.

Because of the suggested importance of phenolics in low nutrient environments, it is likely that species dominating in heath communities contain higher concentrations of phenolics than in meadow communities, because conservation of N within these sites is likely of greater importance (Horner et al. 1988; Aerts & Chapin 2000). In systems characterized by low nutrient availability, such as in subarctic tundra, plants producing litter with high concentrations of phenolic compounds can play an important role through affecting the belowground decomposer system and ultimately nutrient cycling. Low nutrient availability is recognized as a major factor leading to phenol production in plants (Kraus et al. 2004), and several studies have reported that phenols increase in plant tissue with increasing environmental stress (Muller et al. 1987; Herms & Mattson 1992; Northup et al. 1995b).

I expect large differences in the production of phenolic compounds between plants growing in the two vegetation types and across different elevations, because both these factors should affect soil nutrient availability. Responses of phenolic concentrations in plant litter to elevation may occur both across species (i.e., species adapted to different elevations produce different concentrations of polyphenolics) and within species (i.e., phenotypes or genotypes within species vary their phenolic production with elevation). How this variation is expressed across an elevational gradient for each of two contrasting subarctic vegetation types is the focus of this study.

My objective was to study the variation in phenol concentrations and protein precipitation capacity of phenolics in plant litter across a gradient of elevation (and thus temperature) for tundra plants in two distinct vegetation types; heath and meadow, in subarctic Sweden, by testing the following hypotheses:

1) Both total and astringent phenolic compounds will have a higher concentration in litter with increasing elevation as an effect of decreasing temperatures and lower nutrient availability.

2) Plants in heath vegetation will produce litter with higher concentrations of phenolics than those in meadow vegetation. A larger proportion of these phenolics are expected be precipitating protein.

3) Total phenolic content and protein precipitation will increase with elevation both within-species (i.e., any given species will have more phenolics of both types at a higher elevation) and across-species (i.e., species that dominate at higher elevations will produce more phenolics with more astringent properties than those that dominate at lower elevations).

Although many studies have been performed on phenolic variation within species and across environmental gradients (e.g. Muller et al. 1987; Northup et al. 1998; Kraus et al. 2004), no studies have analysed the relationship between phenolics in subarctic species and elevation. As nutrient availability is low and climate (notably temperature) is expected to change most rapidly

in the arctic through global warming (IPCC 2007), climate-driven changes in phenolic production of tundra plants can be expected to have important implications for decomposition rates and nutrient turnover. By addressing these three hypotheses I hope to advance the understanding of the potential role of phenolic production by tundra plants on nutrient cycling.

Material and methods

Study site

This study was performed on the north-east facing slope of Mt Suorooaivi (1193 m), located approximately 20 km south of Abisko, Sweden (68°21'N,18°49'E). The monthly mean air temperature in July 2008, at 400, 700 and, 1000 m.a.s.l. on the slope is 13.3 °C, 12.4 °C and 10.6 °C, respectively. The corresponding temperature in August at these elevations is 9.6 °C, 8.2 °C and 6.3 °C, respectively. The bedrock consists of salic igneous rocks and quartic and phyllitic hard schists. Two types of vegetation, heath and meadow, grow in a mosaic on the slope, with meadow commonly found in depressions. The heath is characterized by ericaceous dwarf-shrubs such as *Vaccinium vitis-idaea* L. and *Empetrum hermaphroditum* Hagerup, and *Betula nana* L. Meadow vegetation is characterized by graminoids such as *Deschampsia flexuosa* (L.) Krin and *Anthoxanthum odoratum* L., sedges such as *Carex bigelowii* Torr ex Schwein and herbs such as *Trollius europeaus* L. and *Solidago virgaurea* L.

In September 2007 four replicate plots (2 m^2) were set up on both heath and meadow vegetation, at every hundred meters along an elevational gradient ranging from 500 to 1000 m.a.s.l., generating 24 plots on each vegetation type – a total of 48 plots. The 500 m site is situated in open birch forest, the site at 600 m is located immediately above the forest line, and the 700-1000 m sites are situated above the treeline.

Litter and soil sampling

The study was performed on litter from dried leaves of 13 common subarctic species. In the meadow the species studied were *Bartsia alpina* L., *Betula pubescens* ssp. *czerepanovii* (N. I. Orlova) Hämet-Ahti, *Carex bigelowii* Torr ex. Schwein, *Carex saxatilis* L., *Carex aquatilis ssp. stans* (Drej.) Hultén, *Geranium sylvaticum* L., *Salix polaris* Wahlenb., *Sibbaldia procumbens* L., *Solidago virgaurea* L., *Trollis europaeus* L. In the heath the species were *B. pubescens* ssp. *czerepanovii*, *Betula nana* L., *C. bigelowii*, *Empetrum hermaphroditum* Hagerup, and *Vaccinium vitis-idaea* L. Litter was sampled from three to four common species in each plot (Appendix 1) during September 2008, which is when most fresh litter is produced.

To compare the sites for total carbon (C), N, C:N ratios, pH, and mineral N (NH_4^+) and P (PO_4^{3-}), soil samples were collected over 24-27 June 2008. Several (≥ 5) 4.5 cm diameter soil cores were collected from each plot to a depth of 5 cm in the mineral soil layer. Sufficient cores

were collected to ensure a minimum of 0.5 l of both mineral soil and humus; all cores within a plot were separated into humus and mineral soil, and bulked. Samples were brought back to the laboratory and kept at 2 °C for a maximum of 24 h before they were passed through a 4 mm sieve. Measurement of pH was made on 6 g fresh weight of humus and 10 g fresh weight of mineral soil for each sample shaken over night in 50 ml DI water. To determine mineral N and P concentrations on each sample, a subsample of 5 g fresh weight humus or 10 g fresh weight mineral soil was extracted in 80 ml 1 M KCl. The KCl extractable concentrations of NH_4^+ and PO_4^{3-} were determined by colorimetry on an AutoAnalyser III (SEAL Analytical, Kontram OmniProcess AB, Sweden). Concentrations were calculated as mg g^{-1} soil dry weight. A subsample of soil from each plot was dried (70 °C, 3 days) and ground on a ball mill (Retsch, MM 301) and the total C and N content was analysed using a Perkin-Elmer 2400 Series II, CHNS/O-Analyzer. For each variable (pH, NH_4^+, PO_4^{3-} and C:N ratios) the mean and SE for the four replicate plots on heath and meadow, respectively, at each elevation was calculated.

Laboratory analysis

For each litter sample a 300 mg ground subsample was soaked in 100 mL of deionized water (DI) and shaken for 24 hours. Samples were filtrated firstly through coarse filters and then filter-sterilized through 0.2 µm disposable filters under vacuum. Extracts were split into two 50 mL tubes and stored in freezer.

The Prussian blue technique (Stern et al. 1996) was used to analyse total phenolic content of the litter extracts. All samples were diluted either 10 times (*B. alpina*, *C. aquatilis* ssp. *stans*, *C. bigelowii*, *C. saxatilis*) or 50 times (*T. europaea*, *G. sylvaticum*, *S. procumbens*, *S. virgaurea*, *S. polaris*, *B. pubescens* ssp. *czerepanovii*, *E. hermaphroditum*, *B. nana*, *V. vitis-idaea*) due to differences in phenolic content.

The protein complexation ability of each litter extract was quantified using a method where external protein is added to the litter extract, which enables measurement of the total protein precipitation capacity at saturation. This approach has some similarities with that of Hagerman (1987), though with some differences in the implementation. Instead of measuring ring formation, this method quantifies the amount of residual protein after full complexation to tannins in the extract. From each extract, 4.5 mL of liquid was transferred to two 15 mL centrifuge tubes in which either 0.5 mL of Bovine Serum Albimun (BSA; Sigma chemicals) or 0.5 mL of DI were added. BSA standards were created (0, 20, 40, 60, 80 and 100 ppm) in order to estimate soluble protein concentrations in each extract. When plotting the BSA content of known standard (y-axis) against absorbance (x-axis) a linear equation is assessed, from which absorbance in the extracts then can be estimated. Tubes were vortexed and left to sit for two hours. Given the initial sterile conditions, it is assumed that this period of time was insufficient to allow any microbial growth to interfere with the measurement. After 2 hours of complexation, each sample was centrifuged for 10 minutes (3000 rpm), causing Phenol-protein complexes to form a pellet at the bottom of each tube. The protein concentration of the supernatant was then

measured and, through subtraction, the quantity of protein removed via complexation was calculated. The protein concentration of the supernatant was measured by pipetting 0.4 mL into a glass screw top test tube containing 3.6 mL of DI water, followed by 1 mL of Bio-Rad reagent. Absorbance was measured at 595 nm using a spectrophotometer. The initial concentration of BSA created in each sample tube was 100 ppm BSA, which saturated the complexation capacity of most extracts. However, litter extracts from three species (*G. sylvaticum*, *V. vitis-idaea* and *B. nana*) complexed 100% of protein at this concentration. Thus, a 1000 ppm BSA concentration was created following the same procedure as previously described, except with the higher BSA concentration and a diluted (1:4) Bio-Rad reagent. One species, *G. sylvaticum*, was still not saturated at this point and had to be measured with a 2000 ppm BSA concentration. The difference between reference tube and supernatant was calculated in order assess the net absorbance. These values were transformed to protein precipitation in ppm using the linear equation generated from the standard calibration curve. Subtracting this value from added protein provide an estimate of the amount of total precipitated protein within the extract.

Statistical analysis

For each variable (i.e., mean phenolic content, protein precipitation capacity and protein per phenol), the average of all species for each plot was calculated to provide an average value for each plot, as described by Wardle et al. (2009). This data was then analyzed using two-way ANOVA to test for the effect of vegetation type and elevation (and their interaction) on the entire data set. The effect of elevation on each variable was then analysed separately for each species that occurred on four or more elevations, by using one-way ANOVA. This enabled us to look at within-species variation in relation to elevation. For all ANOVAs, whenever significant effects of elevation and significant interactive effects between elevation and vegetation type were found, post-hoc comparisons were made using Tukey's HSD at $P<0.05$. To analyse variation at the across-species level, the mean elevation that each species occurred in and the mean value for each of the response variables (phenolic content, protein precipitation capacity and protein per phenol) across all elevations was calculated for that species. Linear regression was then used to test for the relationship of each response variable with elevation with each species serving as an independent data point, as described by Wardle et al. (2009). SPSS (Version 17.0) was used for ANOVA and STATISTIX for the linear regression.

Results

Soil properties

Mineral N and P concentrations of the humus layer decreased with increasing elevation on the heath sites (Table 1), but did not show consistent unidirectional relationships with elevation for

the meadow sites. Soil pH (in both the humus and mineral soil layers) was significantly higher on the meadow compared to the heath sites (Table 1). For the heath site pH generally increased with elevation but no clear trends with elevation occurred for the meadow sites. The soil C:N ratio was higher on the heath relative to the meadow sites, and for both sites was generally greater at lower elevations (Table 1). The humus depth increased with elevation on the heath but not the meadow, and was greater on the heath (data not presented).

Table 1 *Soil components: (mean of four plots ± SE) in humus and mineral soil from two vegetation types, heath and meadow, across an elevational gradient. Within each vegetation and soil type, values marked with the same letters are not significantly different at P = 0.05 (Tukey's HSD).*

		Elevation (m.a.s.l.)					
		500	**600**	**700**	**800**	**900**	**1000**
Meadow							
C:N	humus	18.37±0.92 (a)	15.71±0.45 (b)	14.94±0.45 (b)	15.45±0.79 (b)	16.15±0.32 (b)	16.10±0.41 (b)
NH_4^+ *(mg g-1 DW)*	humus	0.027±0.003 (ab)	0.063±0.039 (ab)	0.043±0.003 (ab)	0.009±0.003(c)	0.054±0.015 (a)	0.019±0.002 (bc)
PO_4^- *(mg g-1 DW)*	*humus*	0.012±0.003 (a)	0.006±0.001 (ab)	0.009±0.001 (a)	0.006±0.001 (ab)	0.014±0.004 (a)	0.004±0.001 (b)
pH	humus	5.90±0.15 (b)	5.34±0.20 (c)	5.31±0.07 (c)	5.49±0.06 (c)	6.37±0.11 (a)	5.49±0.06 (c)
	mineral soil	5.84±0.09 (a)	5.17±0.15 (bc)	4.91±0.08 (c)	5.42±0.03 (b)	6.01±0.09 (a)	5.13±0.15 (bc)
Heath							
C:N	humus	31.88±1.39 (a)	23.71±1.36 (b)	29.21±0.51 (a)	30.61±0.84 (a)	22.58±1.44 (b)	24.19±1.26 (b)
NH_4^+ *(mg g-1 DW)*	humus	0.024±0.002 (a)	0.021±0.002 (a)	0.009±0.001 (b)	0.011±0.004 (b)	0.004±0.001 (c)	0.005±0.001 (c)
PO_4^- *(mg g-1 DW)*	humus	0.037±0.005 (a)	0.009±0.001 (c)	0.025±0.003 (ab)	0.017±0.002 (b)	0.007±0.002 (cd)	0.005±0.001 (d)
pH	humus	4.49±0.07 (c)	4.61±0.10 (c)	4.56±0.10 (c)	4.54±0.03 (c)	5.34±0.21 (a)	4.98±0.10 (b)
	mineral soil	4.33±0.07 (c)	4.46±0.11 (bc)	4.54±0.09 (bc)	4.51±0.06 (bc)	5.64±0.25 (a)	4.92±0.29 (a)

Overall relationship of phenolics with elevation and vegetation type

When data from all species in each plot were averaged and analysis performed on the entire data set, the total phenol content, protein precipitation capacity and the ratio of protein precipitation to phenols were all significantly affected by vegetation type and elevation, and there was a significant interaction between the two for all response variables (Table 2). Total phenol concentration decreased with elevation in the meadow vegetation and was least for the highest elevations (Fig 1a). In the heath vegetation, the top and bottom elevations had the lowest amounts of phenols (Fig 1b). Total phenol content was greater in heath vegetation than in meadow vegetation, with the exception of the two lowest elevations.

Protein precipitation capacity was generally lower in litter from meadow vegetation than in heath vegetation, though litter from the lowest two meadow elevations precipitated the most protein of all sites (Fig 1c, d). This was mainly due to the effects of one species (*G. sylvaticum*, see Appendix 1). Protein precipitation was very low at other elevations on the meadow. There was a general trend of decline in protein precipitation with elevation on the heath.

When the amount of protein precipitated per phenol was considered, the most protein precipitating phenols were found in litter for the bottom two elevations on meadow and the lowest elevation on heath, suggesting a larger proportion of tannins in litter at these elevations (Fig 1e, f). Apart from these two elevations, there was no difference in protein:phenol ratio with elevation for either of the vegetation types.

Table 2 *Two-way ANOVA results (F- and P-values) testing for the effect of vegetation type (heath versus meadow) and elevation on total phenolic content, protein precipitation capacity and precipitated protein per phenol in plant litter, with data from all species in each plot being averaged before data analysis.*

	ANOVA		
	Vegetation type (V)	Elevation (E)	V x E interaction
Total phenol content	**240.6 (<0.001)**	**30.2 (<0.001)**	**60.8 (<0.001)**
Protein precipitation capacity	**21.4 (<0.001)**	**58.8 (<0.001)**	**41.6 (<0.001)**
Protein:phenol	**52.9 (<0.001)**	**68.4 (<0.001)**	**35.9 (<0.001)**

Values in boldface indicate statistical significance at $P \leq 0.05$. Degrees of freedom are 1, 36 for V, 5, 36 for E and 5, 36 for E x V

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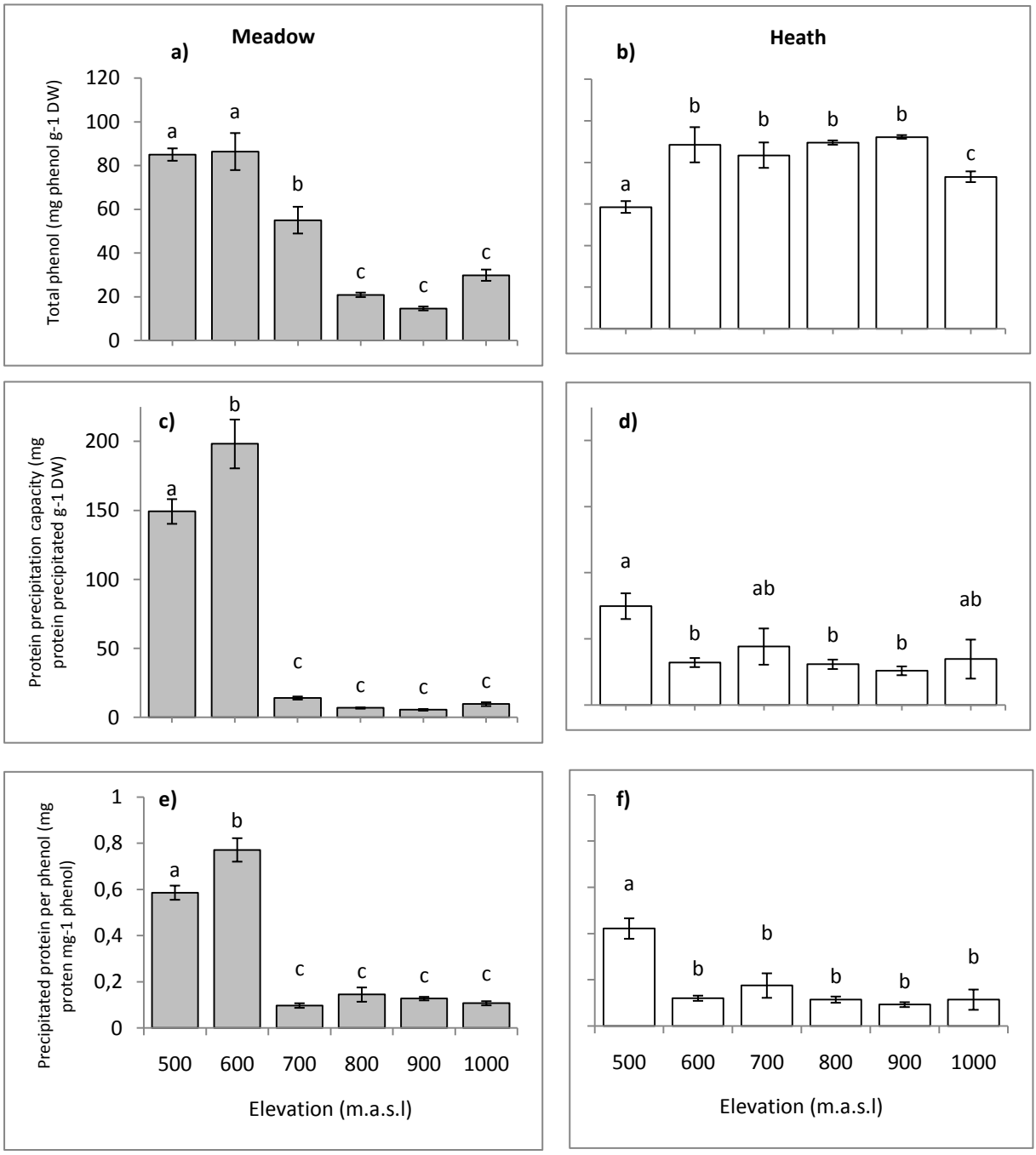

Fig 1 *Total phenol content (a and b), protein precipitation capacity (c and d) and protein precipitated per phenol (e and f) per elevation for meadow and heath, with data from all species in each plot being averaged before data analysis. Error bars = SE. Within each panel bars topped by the same letter are not significantly different at P < 0.05 (Tukey's HSD) following two-way ANOVA (results in Table 2).*

Between-species differences

At the between-species level (when each species was represented as a single data point) there was a significant negative relationship of both total phenolics and protein precipitation capacity with elevation; species which dominated at higher elevations produced fewer total phenolics, and phenolics with less protein precipitating capacity than species that dominated at lower elevations (Fig 2 a,b). Across species, the protein to phenol ratio was not significantly related to elevation (data not presented).

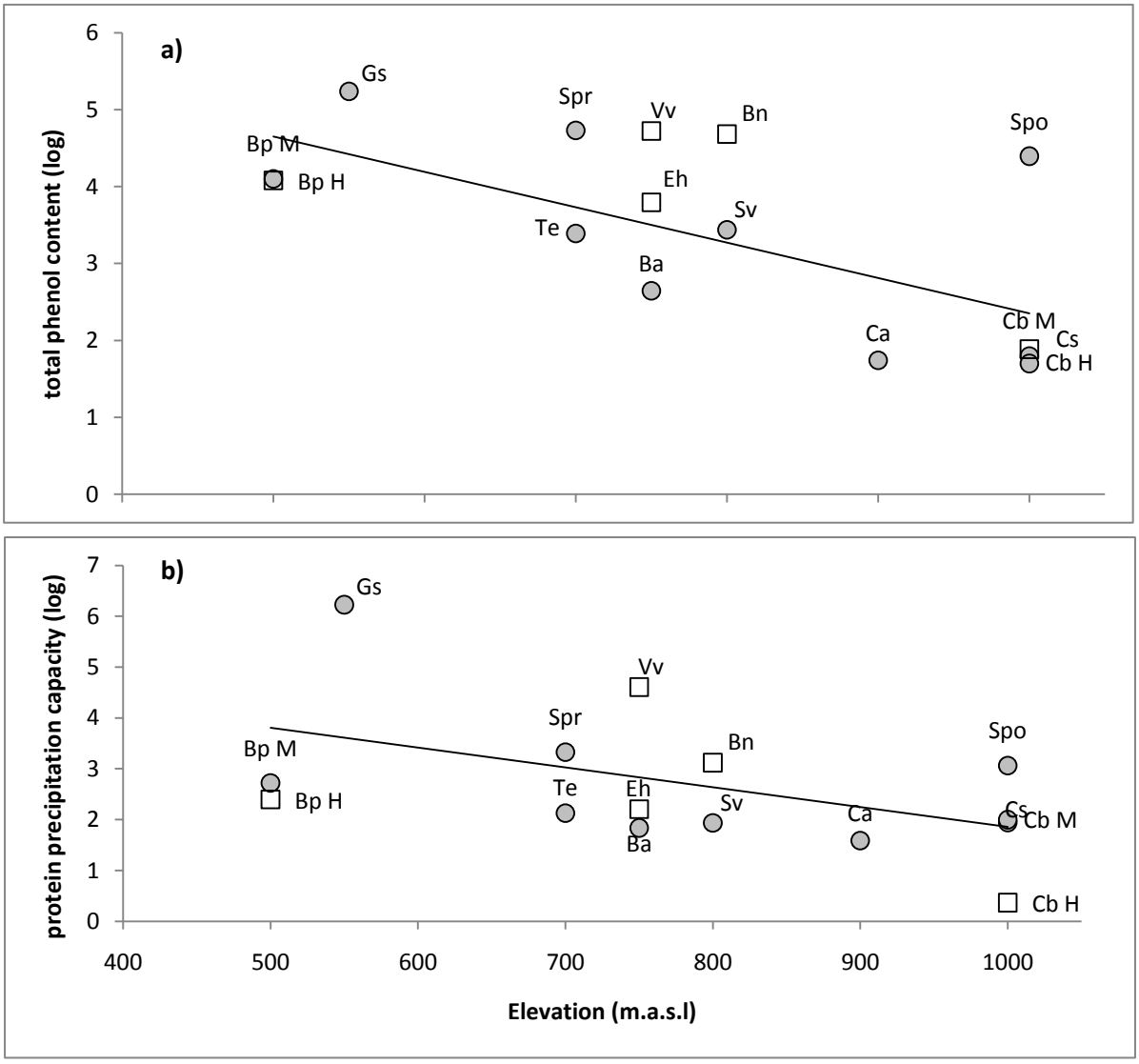

Fig 2 *Linear regression of total phenol content (a) and protein precipitation capacity (b) against elevation, with each species representing an independent data point. For each species the mean elevation at which that species occurs across the gradient, and the value for phenolic or protein precipitation capacity used is the mean for that species across all elevations. The* R^2 *value between elevation and (log-transformed) total phenol content is 0.421 (P < 0.001) and between elevation and protein precipitation capacity is 0.236 (P = 0.027). Filled circles indicate meadow and empty squares heath. Ba – Bartsia alpina; Bn – Betula nana; Bp – Betula pendula ssp. czerepanovii; Ca – Carex aquatilits ssp. stans; Cb – C. bigelowii; Cs – C. saxatilis; Eh – Empetrum hermaphroditum; Gs – Geranium sylvaticum; Spo – Salix polaris; Spr – Sibbaldia procumbens; Sv – Solidago virgaurea; Te – Trollius europaeus; Vv – Vaccinium vitis-idaea. M = meadow, H = heath.*

Within-species differences

Species that were present at more than four elevations were analysed to assess within-species responses to elevation. These species included *E. hermaphroditum*, *V. vitis-idaea* and *B. nana* from the heath, and *T. europaea* and *B. alpina* from the meadow. Elevation had a significant effect on phenol content, protein precipitation capacity and protein precipitated per phenol ratio for *E. hermaphroditum* and *V. vitis-idaea* (Table 3). Astringent phenolics in *E. hermaphroditum*, as measured by protein precipitation capacity, clearly decreased with increasing altitude (Table 2; Fig 3). Phenol content and protein precipitated per unit phenol also varied significantly with elevation, but the decreasing trend was not as strong. The phenol content of *V. vitis-idaea* increased with increasing elevation (Table 3; Fig 4). However, protein precipitation capacity and protein precipitated per unit phenol did not follow the same trend, and the highest values for both variables were at the lowest elevation. The phenol content in *T. europaeus* litter was also significantly affected by elevation (Table 3), but did not show a simple relationship with elevation (Fig 5). Protein precipitation capacity and precipitation per unit phenol of this species were both unresponsive to elevation. No response variables for the other two species (*B. nana* and *B. alpina*) showed any relationship with elevation.

Table 3 *One-way ANOVA results (F- and P-values) testing for the effect of elevation on total phenolic content, protein precipitation capacity and precipitated protein per phenol on five litter types that were present at all locations across the elevational gradient.*

	Total phenol	Protein precipitation capacity	Protein precipitated per phenol
Empetrum hermaphroditum	**4.04 (0.012)**	**8.45 (<0.001)**	**4.03 (0.013)**
Vaccinium vitis-idaea	**13.28 (<0.001)**	**3.10 (0.034)**	**14.54 (<0.001)**
Betula nana	1.78 (0.186)	1.37 (0.291)	1.28 (0.321)
Trollius europaeus	**4.40 (0.015)**	0.36 (0.834)	1.54 (0.242)
Bartsia alpina	0.94 (0.455)	1.18 (0.361)	0.73 (0.553)

Values in boldface indicate statistical significance at $P \leq 0.05$. Degrees of freedom for Empetrum hermaphroditum and Vaccinium vitis-idaea are 5, 18, for Bartsia alpina are 3, 11 and for Trollius europaeus and Betula nana are 4, 15.

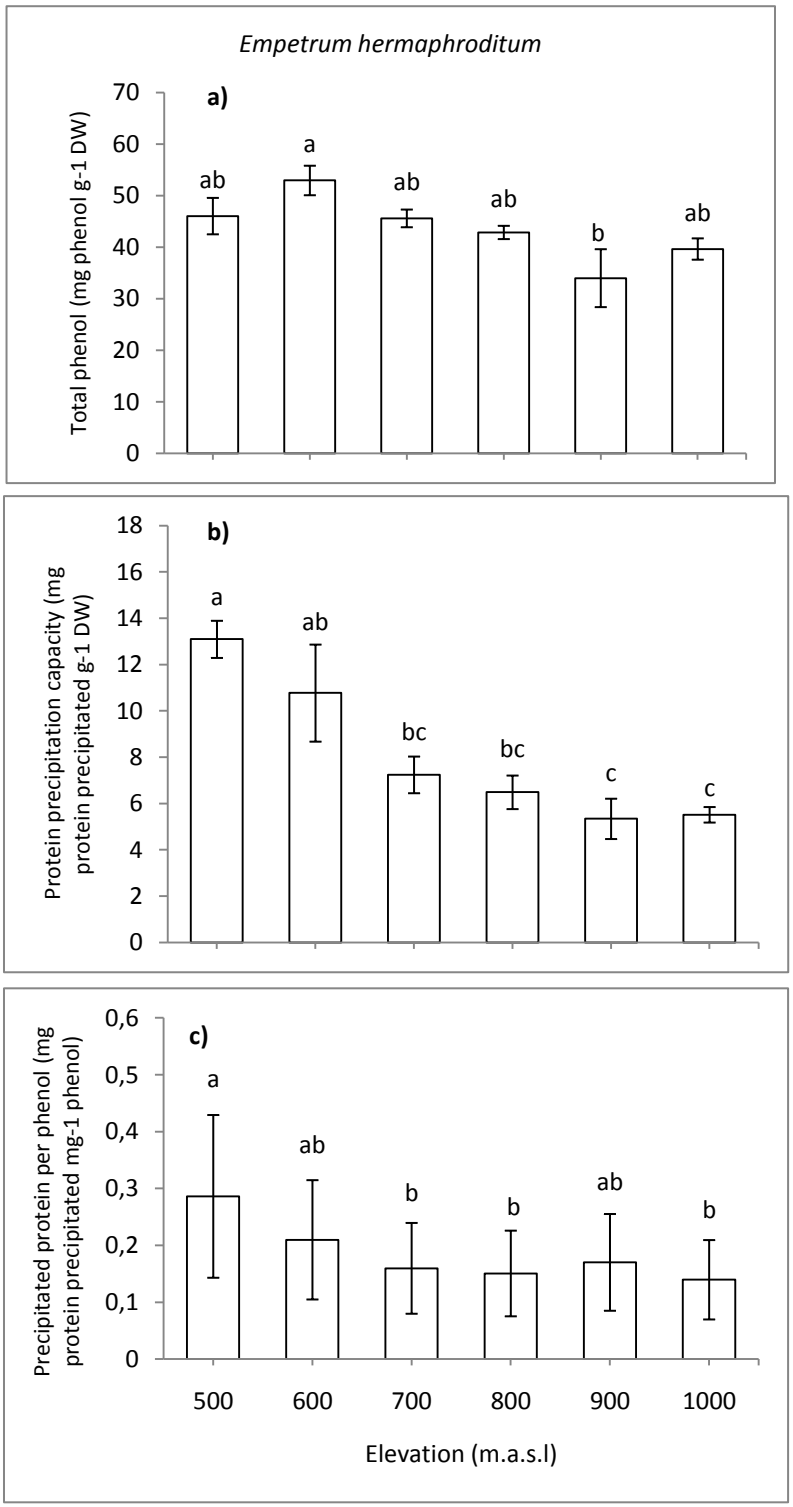

Fig 3 *Total phenol content (a), protein precipitation capacity (b) and protein precipitated per phenol (c), in Empetrum hermaphroditum litter in relation to elevation. Bars represent plot means. Error bars = SE. Within each panel bars topped by the same letter are not significantly different at P < 0.05 (Tukey's HSD) following two-way ANOVA (results in Table 3).*

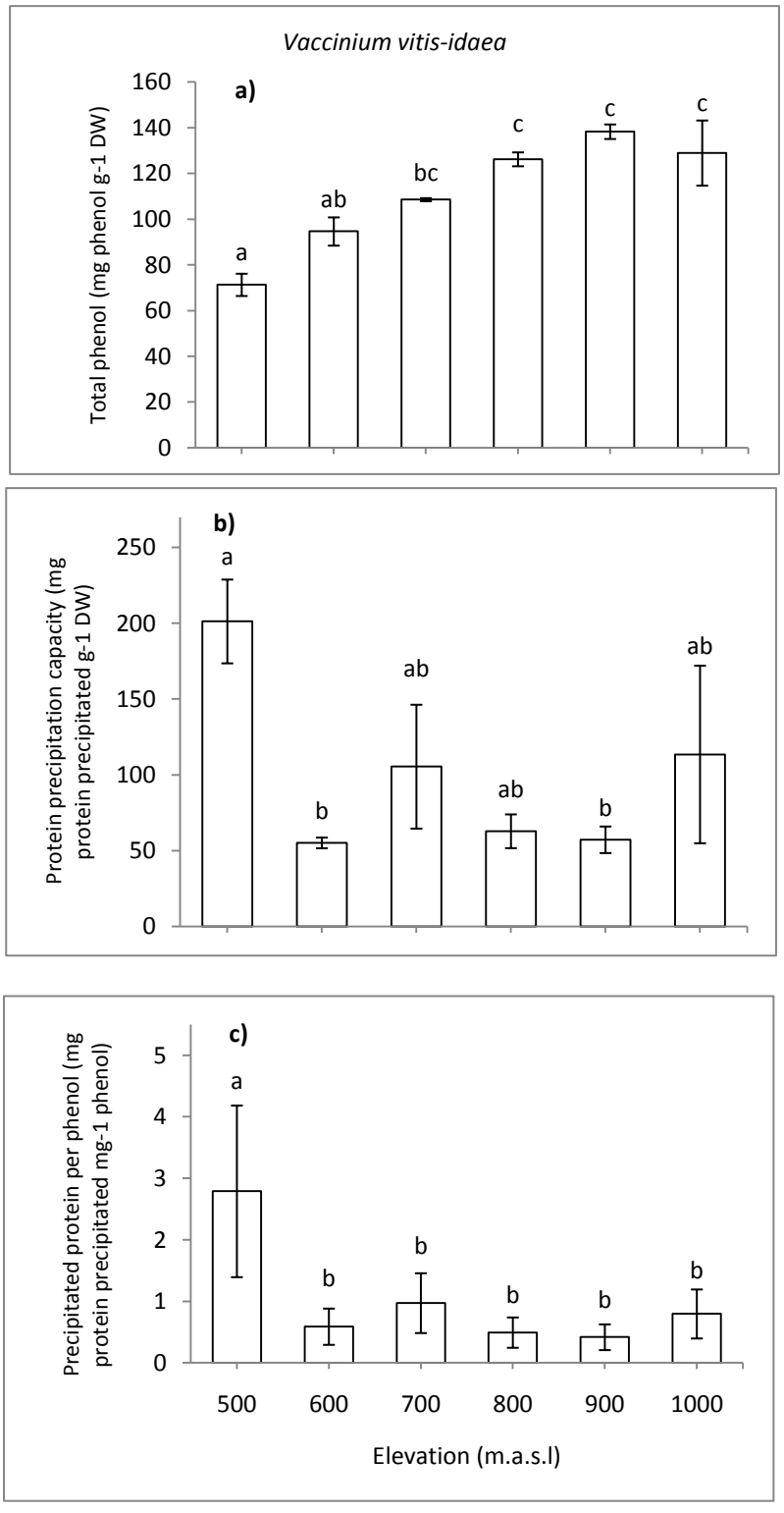

Fig 4 *Total phenol content (a), protein precipitation capacity (b) and protein precipitated per phenol (c) in Vaccinium vitis-idaea litter in relation to elevation. Bars represent plot means. Error bars = SE. Within each panel bars topped by the same letter are not significantly different at P < 0.05 (Tukey's HSD) following two-way ANOVA (results in Table 3).*

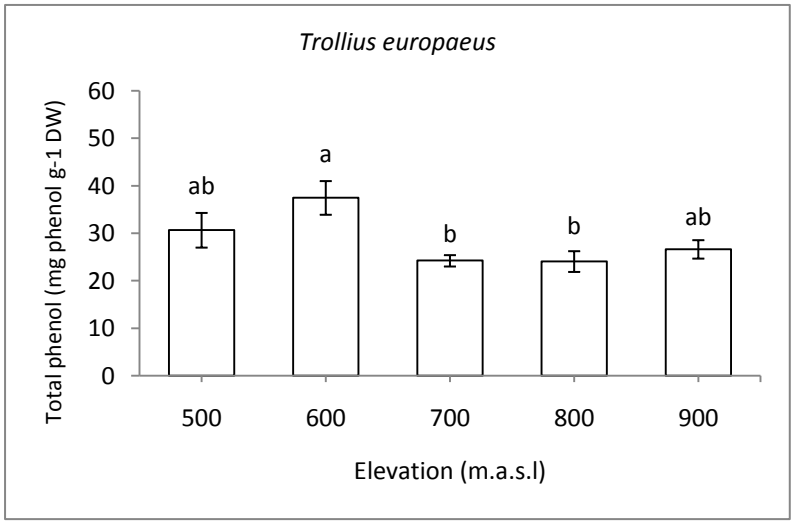

Fig 5 *Total phenol content in Trollius europaeus litter in relation to elevation. Bars represent plot means. Error bars = SE. Within each panel bars topped by the same letter are not significantly different at P < 0.05 (Tukey's HSD) following two-way ANOVA (results in Table 3).*

Discussion

Relationship of phenolic properties with vegetation and type elevation

This study found a strong impact of both elevation and vegetation type on total phenolic content and protein precipitation when averaged across all species for each plot (Fig. 1). I hypothesized that heath vegetation would have higher total phenolic concentrations compared to meadow vegetation and that total foliar phenolic concentration would increase with elevation for both vegetation types, due to a change in nutrient availability across the gradient. My results show that NH_4^+ and PO_4^{3-} concentrations decreased with elevation in heath as predicted, whereas nutrient concentrations did not show a unidirectional response to elevation in meadow sites. The data show higher total amounts of phenols in the heath compared to the meadow communities, providing strong support for my second hypothesis. However, contrary to my first hypothesis, phenols decreased with elevation in the meadow, while in the heath the lowest and highest sites contained the lowest concentrations of phenols. As nutrient availability is generally higher at low compared to high elevations (Sveinbjörnsson et al. 1995; Hart and Perry 1999; Huber et al. 2007), the results are inconsistent with previous studies that have suggested a negative relationship between phenolic content and nutrient availability (Bryant et al. 1987, Herms & Mattson 1992, Northup et al. 1998) or a dominance of tannin-rich species on infertile soils (Northup et al. 1999). My results are instead at least partially consistent with the study of Hättenschwiler et al. (2003) in montaine rainforests in Hawaii, for which plants dominating at the most nitrogen limited sites (but not the most phosphorus limited sites) did not produce litter with more phenolics than those on non-nutrient limited sites.

Phenolics are a diverse group of compounds that vary greatly in their impact on proteins and nutrient cycling (Schnitzer et al. 1984; Schofield et al. 1998; Fierer et al. 2001). Tannins are a highly active fraction of this group and are recognised for their ability to

precipitate protein and form phenol-protein complexes (Hagerman 2002). I hypothesised that protein precipitation capacity would be higher in heath vegetation compared to meadow vegetation, and would increase with elevation and thus increasing environmental stress for both vegetation types. However, the results show a general trend of decline in protein precipitation with increasing elevation for both vegetation types. As was found for total phenolic concentrations, the overall protein precipitation capacity was higher in heath, except for the two lowest elevations where the meadow had a significantly higher protein precipitation capacity than all other sites in this study. As such, the higher protein precipitation capacity of both heath and meadow litter at lower elevations points to a greater capacity to retain nitrogen and prevent its release to the soil solution at these sites. This result is only partially consistent with previous studies pointing to dwarf shrubs being effective in producing polyphenol complexes that reduce soil N availability (Nilsson and Wardle 2005). The soil C:N ratio was higher and NH_4^+ concentrations were lower on the heath sites for which protein precipitation was higher. This is consistent with earlier findings that astringent phenols are associated with conditions of low soil N availability (Schimel et al. 1998; Steltzer & Bowman 1998). However, these results are contradicted by the lowest two elevations on the meadow which had the highest proportion of astringent phenolics in leaf litter but higher NH_4^+ concentrations and lower C:N ratios compared to heath.

The reactivity of polyphenolic compounds is an important aspect in understanding the phytochemistry of subarctic plants. The large differences in tannin production (i.e., amount of protein precipitated) both across elevations and between the vegetation types suggest that spatial variation in environmental conditions across the tundra landscape is a major determinant of plant allocation to the production of these compounds. This is consistent with previous studies suggesting that plants may be able to regulate the proportions of astringent phenols in their litter according to current nutrient status, competition and/or herbivore frequency (Horner et al. 1988). Even though the heath plants generally contained greater concentrations of phenols than did the meadow plants, the meadow plants produced litter that was more efficient in precipitating proteins, and so the ratio of protein precipitated per unit phenol for both vegetation types were similar, except for on the lowest elevations where the ratio was highest for the meadow. As such, contrary to my predictions which were based on earlier studies (e.g. Muller et al. 1987; Kraus et al. 2004), the phenols produced on the more nutrient rich meadow were more effective at precipitating proteins on a per unit phenol basis than those produced on the heath site.

Between-species differences

I found a negative relationship of both phenol concentration and protein precipitation with elevation across all species, when each species represented a single data point (Fig 2), meaning that species dominating at higher elevations generally produced less phenolics with less astringent properties than those that dominated at lower elevations. As species that dominate at lower elevations for both heath and meadow invest more in both total phenol production and those phenols that complex nitrogen, this suggests that lower-elevation species

are the most effective at locking up nitrogen and thereby restricting its availability for use by competing species (Berendse & Aerts 1987; Horner et al. 1988; van Breemen 1993).

Plant species differ in their ability to access nitrogen from proteins precipitated by polyphenols, and this may to some extent be governed by their mycorrhizal associations. In tundra ecosystems, some studies have suggested that these complexes can exclusively be utilized by the ericoid mycorrhizae present in *Vaccinium* species and *E. hermaphroditum*, and sometimes by the ectomycorrhizal fungi of *B. nana* (Bending & Read 1996a,b; Tybirk et al. 2000; Clemmensen et al. 2006). This suggests that some species that produce phenolics with a high protein precipitation capacity may also be able to access nitrogen from these complexes. Plants that produce organic matter low in nutrients and high in phenolic content are known to support microbial communities dominated by fungi (Högberg et al. 2007; Eskelinen et al. 2009) that are more conservative in cycling nutrients (Coleman et al. 1983). Ultimately this can in turn be beneficial for plant species that produce litter high in phenolics, through lowering the nutrient supply for competing plant species that have inherently higher relative growth rates (van Breemen 1993; van Breemen & Finzi 1998).

I hypothesised that woody heath species that are known to produce litter high in phenolics, and species that dominate at the highest elevations would contain the highest amount of phenolics and have the highest precipitation capacity. However, the herb *G. sylvaticum*, found on the lower parts of the gradient and in the meadow sites, contained by far the most phenolics with the most astringent properties. This is inconsistent with my expectations, and suggests that *G. sylvaticum* has a secondary chemistry that differs from most forb species that are adapted for fertile sites.

Within-species differences

According to the carbon-nutrient balance hypothesis (Bryant et al. 1987) and the growth-differentiation hypothesis (Herms & Mattson 1992), growth is prioritised over defence when resources are replete. As such, nutrients are primarily used for assimilation of biomass until sources are depleted, at which stage an increasing proportion of C is used for the production of C-based secondary compounds. In this study two dominant heath species, the evergreen dwarf-shrubs *V. vitis-idaea* and *E. hermaphroditum*, appear to have different strategies and differ in the support that they offer for these theories. The species *V. vitis-idaea* clearly invests more C in phenols at higher elevation, while *E. hermaphroditum* had the opposite strategy, investing more in phenols at lower elevations. However, the nature of the phenolic compounds produced by these two species was found to be similarly affected by elevation, as *V. vitis-idaea* produced the highest proportion of tannins at the bottom of the gradient and the tannin levels in *E. hermaphroditum* strongly decreased with increasing elevation. As such, both species strongly contradicted the hypothesised trends, as astringent phenolic production was greatest in the environmental setting in which nutrient availability was greatest. Furthermore, the phenolic content and protein precipitation of *B. nana*, *T. europaeus* and *B. alpina* were non-responsive to elevation; with exception of total phenol content in *T. europaeus*, which varied inconsistently over the gradient. As such, these results point to

considerable variation among species in how they respond to elevation in terms of phenol production.

Only a small part of the *E. hermaphroditum*'s dry weight is constituted by phenolic compounds, in this study 3-5 %, while *V. vitis-idaea* contains 7-14 % and *B. nana* contains 7-13 % (Appendix 1). However, previous studies have shown that one of the main phenolics produced by *E. hermaphroditum* is the highly allelopathic phenolic compound batatasin-III (e.g. Nilsson 1994, Tybirk et al. 2000; Wallstedt et al. 2005) which exhibits strong effects on biological processes even at very low concentrations (Nilsson et al. 1998. This highlights the importance of distinguishing between the total amounts of phenolics a plant produces versus the composition of these phenolics. Different phenolic fractions can differ considerably in the strength of their effects on various processes, such as herbivory, litter decomposition, organic matter quality and nutrient cycling, or direct allelopathic effects on other species (Schimel et al. 1998; Bais et al. 2003; Meier & Bowman 2008).

Conclusions

The results of this investigation show that both the production of total phenolics and their protein precipitation capacity can decrease with elevation, and at both the within-species and across-species levels. As nutrient availability is generally less at higher elevations, my results strongly contradict theories which predict that phenolic or tannin production in subarctic plants is regulated by nutrient availability. As such, they show that plants growing at lower elevations often produce not just more total phenols but also those phenols that can complex nitrogen. Controlling the fate of N in soils may be potentially more important for plants in high resource sites in which the intensity of resource competition among species may be greater. Also, herbivore pressure is likely to be stronger in more fertile (e.g., lower elevation) sites and greater production of astringent phenolics at these sites may confer greater protection from herbivory. Although there are no other comparable studies from tundra ecosystems, my results are not in accordance with those from many previous studies in other types of ecosystems which have shown a negative relationship between phenols and nutrient availability. They further show that spatial variation in environmental conditions across the tundra landscape is a major determinant of plant allocation to the production of phenolic compounds. Given the tendency of phenolics in my study to be produced in greater amounts at warmer low elevation sites in which nutrient availability is greater, it is plausible that a future increase in nutrient availability at higher elevations that may occur as a result of global warming may favour plants that produce high concentrations of polyphenolic compounds, especially tannins. This may play an important role in shaping the structure of the plant and soil community and may have implications for nutrient availability and plant species coexistence.

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Appendix *Sampled litter: total phenol content, precipitated protein and protein precipitated per phenol. Mean values ±SE*

Meadow

	Elevation *m a s l*	**Total phenol content** *mg phenol g-1 DW*	**Precipitated protein** *mg protein g-1 DW*	**Protein:phenol** *mg precipitated protein mg-1 phenol*
Bartsia alpina	600	12.52±1.85	3.70±1.21	0.31±0.13
	700	14.24±1.03	6.75±4.38	0.49±0.33
	800	13.37±1.39	5.79±1.64	0.43±0.10
	900	12.50±1.74	5.22±1.11	0.42±0.04
Betula pubescens ssp. czerepanovii	500	59.34±10.98	14.18±2.01	0.24±0.02
Carex bigelowii	1000	5.03±0.53	5.98±0.60	1.20±0.20
Carex saxatilis	1000	4.47±0.79	6.37±0.63	1.46±0.30
Carex aquatilis ssp. stans	900	4.70±0.50	3.88±0.52	0.83±0.14
Geranium sylvaticum	500	164.93±21.90	426.31±55.34	2.59±0.22
	600	209.03±45.07	583.50±105.44	2.82±0.33
Salix polaris	1000	68.8±10.26	20.35±4.78	0.23±0.05
Sibbaldia procumbens	700	112.33±16.16	26.82±1.83	0.24±0.03
Solidago virgaurea	800	30.09±0.38	5.91±1.33	0.20±0.05
Trollis europaeus	500	30.68±7.31	7.37±1.14	0.24±0.02
	600	37.50±7.10	7.42±1.49	0.20±0.02
	700	24.24±2.40	6.17±4.40	0.25±0.17
	800	24.10±4.38	8.16±1.29	0.35±0.08
	900	26.65±3.88	7.76±2.52	0.29±0.06

Heath

	Elevation *m a s l*	**Total phenol content** *mg phenol g-1 DW*	**Precipitated protein** *mg protein g-1 DW*	**Protein:phenol** *mg precipitated protein mg-1 phenol*
Betula pubescens ssp. Czerepanovii	500	58.10±8.33	9.99±4.75	0.23±0.05
Betula nana	600	117.64±11.24	30.47±16.45	0.25±0.12
	700	96.08±10.14	19.98±1.87	0.21±0.02
	800	99.28±7.16	23.11±13.31	0.23±0.12
	900	104.27±29.61	15.14±4.07	0.15±0.02
	1000	117.93±4.63	19.54±2.11	0.17±0.02
Carex bigelowii	1000	5.59±0.32	0.44±0.89	0.08±0.16
Empetrum hermaphroditum	500	46.02±7.06	13.10±1.60	0.29±0.02
	600	52.94±5.72	10.77±4.19	0.21±0.10
	700	45.58±3.43	7.24±1.58	0.16±0.04
	800	42.85±2.57	6.49±1.45	0.15±0.03
	900	33.99±11.23	5.34±1.74	0.17±0.07
	1000	39.64±4.13	5.52±0.67	0.14±0.01
Vaccinium vitis-idaea	500	71.32±9.73	201.22±55.28	2.78±0.54
	600	94.65±12.35	55.15±7.02	0.59±0.10
	700	108.58±1.35	105.43±81.72	0.97±0.76
	800	126.23±6.06	62.83±22.25	0.49±0.16
	900	138.29±6.36	57.20±17.49	0.42±0.15
	1000	128.97±28.48	113.48±117.14	0.80±0.64

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