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# Chemical properties of plant litter in response to elevation: subarctic vegetation challenges phenolic allocation theories

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**Running headline: Shifts in leaf litter phenolics with elevation**

## Summary

1. Several theories predict that increasing stress (e.g., decreasing nutrient availability or decreasing temperature) should result in higher amounts of plant phenolic compounds both at the interspecific and intraspecific levels. Further, several theories predict that plant phenolics are major drivers of plant-soil feedbacks whereby they influence litter decomposition rates and the return of nutrients to plants.

2. We investigated the potential influence of shifts in abiotic factors on litter phenolic properties using an elevational gradient in northern Sweden, for which temperature and soil fertility decline with increasing elevation. The system consists of two vegetation types; heath, (associated with low soil fertility), and meadow (associated with higher fertility) which occur across the entire gradient.

3. We hypothesized that total phenolics, tannins, and protein complexation capacity (PCC) of leaf litter would increase with elevation within and among plant species. We further hypothesized that at the whole-plot level (using community weighted averages) these properties would be higher in heath than meadow, and that phenolic properties for meadow vegetation would show stronger responses to elevation than for heath.

4. We measured phenolic properties in leaf litter for 13 species from both vegetation types across an established elevational gradient (500-1000 m) in Swedish subarctic tundra.

5. Contrary to our hypotheses, different species showed highly contrasting responses in their phenolic characteristics to elevation. At the across-species level, total phenolic content in litter decreased with elevation. At the whole plot level, tannin concentrations were higher for the heath

than for the meadow, whereas total phenolics and PCC did not differ. However, consistent with our hypothesis, our results showed that phenolic properties were more responsive to elevation for the meadow compared to the heath, as a consequence of greater species turnover for the meadow.

6. Our results are inconsistent with theories predicting higher plant phenolic concentrations with increasing environmental stress or decreasing nutrient availability. They also provide evidence that across abiotic gradients in the subarctic tundra there are large shifts in litter phenolic properties (including those that are able to complex protein), and highlight that the direction and strength of such shifts may differ greatly among vegetation types.

**Key-words:** carbon nutrient balance hypothesis, decomposition, litter chemistry, litter feedback, plant defence theory, protein complexation capacity, condensed tannins, tundra**.**

## Introduction

Phenolics are a broad class of carbon-based secondary metabolites that can protect plants against photo-damage (Close & McArthur 2002) and are well known to defend plants against herbivores or pathogens by reducing their palatability and interfering with the enzyme activities of their enemies (Coley, Bryant & Chapin 1985; Joanisse *et al*. 2007). Further, phenolic compounds persist in plant tissues following senescence, and have been found to have important litter afterlife effects on decomposition, microbial nutrient immobilization and nutrient availability (Northup *et al*. 1995; Hättenschwiler & Vitousek 2000; Cornelissen *et al*. 2004). One class of phenolics, tannins, are defined by their reactivity with proteins, and can inhibit microbial enzyme activity (Joanisse *et al.* 2007) and form recalcitrant tannin-protein complexes (Hagerman 1987; Hättenschwiler & Vitousek 2000; Joanisse *et al*. 2009), which can reduce rates of decomposition, nitrogen (N) mineralization (Northup *et al*. 1995) and the availability of N to plants (Kraus, Dahlgren & Zasoski 2003). These effects of litter phenolics on soil processes are proposed to control the supply rate of nutrients back to plants, and therefore have feedback effects on plant growth (e.g. Northup, Dahlgren & McColl 1998; Meier & Bowman 2008; Joanisse *et al*. 2009).

In natural environments there is great variability in the biotic and abiotic conditions that plants encounter, and there is much interest in understanding how plant secondary metabolites allow plant species and communities to respond to this variability (Kraus *et al*. 2003a; Barbehenn & Constabel 2011). Several theories have been developed to explain the inter- and/or intra-specific variation in plant phenolic production observed in many natural environments. For instance, the ‘Carbon Nutrient Balance Hypothesis’ (Bryant, Chapin & Klein 1983), the ‘Oxidative Pressure Hypothesis’ (Close & McArthur 2002), the ‘Growth Rate Hypothesis’

(Coley *et al*. 1985), and the 'Protein Competition Model' (Jones & Hartley 1999) all predict that either increasing stress or decreasing nutrient availability should result in higher production of phenolic compounds. However, the generality of these theories and their applicability to contrasting environments have been strongly debated (Hamilton *et al*. 2001; Koricheva 2002; Lerdau & Coley 2002; Stamp 2003). Further, some resource competition theories propose that, as nutrient availability and plant productivity decline, competition for soil resources should become relatively more important than competition for light (Tilman 1985; Huston & DeAngelis 1994). Since green leaf traits are usually strongly related to litter traits (Cornwell *et al*. 2008; Fortunel *et al*. 2009) including chemical traits such as phenolics (Pastor & Naiman 1992; Schweitzer *et al*. 2004, Hättenschwiler, Hagerman & Vitousek 2003), it has been hypothesized that low litter quality and decomposability (Berendse 1994) and a high content of some phenolics (particularly tannins) in litter provides an adaptive advantage for plants in low resource environments through the afterlife effect of those litters on soil fertility (Hättenschwiler & Vitousek 2000; Hättenschwiler *et al*. 2003; Joanisse *et al*. 2009). In support of this, recent studies suggest that the creation of protein-tannin complexes by certain plant species, notably ericaceous shrubs, allows them to exclude competing species, because their ericoid mycorrhizal associates can preferentially access this tannin-complexed organic N (Joanisse *et al*. 2009; Wurzburger & Henrick 2009).

While several studies have investigated potential afterlife effects of phenolics in litter at either the inter- or intraspecific-level along natural abiotic gradients in forested ecosystems (Northup *et al*. 1995; Hättenschwiler *et al*. 2003; Schweitzer *et al*. 2008; Gundale *et al*. 2010), fewer have been conducted in other major types of ecosystems, such as subarctic or tundra ecosystems. In the present study we use an elevational gradient in Swedish subarctic tundra

(Sundqvist *et al*. 2011a, Sundqvist, Giesler & Wardle 2011) to explore whether inter- and intraspecific variation in litter phenolic properties is consistent with several theories predicting how plant phenolic levels and plant-soil feedback effects change across abiotic gradients. Elevational gradients serve as powerful tools for testing how ecological properties and processes respond to changes in temperature, especially when other extrinsic factors are relatively constant (Fukami & Wardle 2005; Körner 2007). As elevation (and thus temperature) has important effects on the soil microbial community, nutrient cycling and nutrient supply rates from the soil (Ruess *et al*. 1999; Sundqvist *et al*. 2011a), they also provide the opportunity for investigating plant litter phenolics in response to these factors.

The Swedish subarctic tundra contains large gradients of elevation across which two functionally contrasting vegetation types co-occur, heath (dominated by dwarf shrubs) and meadow (dominated by herbaceous plants), which are likely to differ greatly in terms of their litter phenolics as well as in the afterlife effects of these compounds. The heath is characterized by lower pH and soil N availability (Björk *et al*. 2007; Eskelinen, Stark & Männistö 2009), and higher soil P availability, than the meadow (Giesler *et al*. 2011; Sundqvist *et al*. 2011a). Previous work on this gradient has shown that for both vegetation types, increasing elevation is associated with an increase in foliar and leaf litter N:P ratios both within and across species, as well as at the whole-community level (see Fig. 1., Table S1 in Supporting Information; Sundqvist *et al*. 2011b). This work has also revealed that other leaf traits sometimes also shift with increasing elevation from those associated with high growth rates and a nutrient acquisitive strategy, toward those associated with slow growth and a nutrient conservative strategy. Further, most leaf and litter traits, and litter decomposability, show a greater responsiveness to elevation for the meadow vegetation (Fig. 1, Table S1; see also Sundqvist *et al*. 2011b) which also has a greater turnover of species with increasing elevation relative to the heath (Sundqvist *et al*. 2011a). With regard to soil properties, available N and P concentrations decline, and fungal:bacterial ratios increase with elevation for the heath, while these properties vary greatly but idiosyncratically with elevation for the meadow (Sundqvist *et al*. 2011a). Therefore, this system enables determination of how litter phenolic properties for a range of species occurring in functionally contrasting vegetation types respond to the same elevational gradient.

In this study system we tested the following four hypotheses: 1) For those species spanning a significant portion of the elevational gradient, we hypothesized that an increase in elevation (and thus a decline in temperature and soil fertility) would be associated with an increase in plant litter concentrations of total phenolics and total tannins, and would exhibit a greater capacity to complex proteins; 2) At the across-species level, we hypothesized that species that dominate at higher elevations will produce litter with higher concentrations of phenolics and total tannins, and exhibit a greater capacity to complex proteins compared to those species that dominate at lower elevations; 3) We hypothesized that the community weighted average concentrations of total phenolics and tannins of the local community (i.e. at the plot-level) would be higher in the infertile heath vegetation than in the meadow vegetation; and 4) We hypothesized that these plot-level measures of phenolic properties would be more responsive to elevation for the meadow than the heath. This prediction is consistent with the greater rate of species turnover with increasing elevation previously shown for the meadow relative to the heath (Sundqvist *et al*. 2011a).

## Methods

### *Study site and litter sampling*

This study was performed on the north-east facing slope of Mt Suorooaivi (1193 m), located approximately 20 km south-east of Abisko, Sweden (68°21'N, 18°49'E). The bedrock consists of salic igneous rocks and quartic and phyllitic hard schists. Measurements of air temperature by data-loggers have confirmed that air temperature decreases with elevation across this gradient (Sundqvist *et al*. 2011a, Fig. S1 in Supporting Information). Two types of vegetation, heath and meadow, grow in a mosaic on the slope, with meadow commonly found in shallow depressions. The heath is characterized by ericaceous dwarf-shrubs such as *Vaccinium vitis-idaea, Empetrum hermaphroditum*, and *Betula nana*. Meadow vegetation is characterized by graminoids such as *Deschampsia flexuosa* and *Anthoxanthum odoratum*, sedges such as *Carex bigelowii* and herbs such as *Trollius europaeus* and *Solidago virgaurea*. The mean soil humus depth across the study system (mean ± SE) is 6.0 (± 0.3) cm and 2.7 (± 0.4) cm for heath and meadow respectively (data derived from Sundqvist *et al.* 2011a). For the heath, the maximum soil humus depth is 7.3 (± 0.4) cm, and the minimum is 4.6 (± 0.4) cm, found at the highest and the lowest elevation, respectively. For meadow, the maximum soil humus depth is 6.8 (± 1.2) cm, and the minimum humus depth is 1.2 (± 0.1) cm, found at the lowest and highest elevation, respectively. Previous work along this study site has revealed that increasing elevation is associated with an increase in foliar and leaf litter N:P ratios, but that this increase is greater for the meadow than for the heath (Sundqvist *et al*. 2011b). It has also been shown in this system that available soil N and P decline with elevation for the heath and vary greatly but idiosyncratically for the meadow (Sundqvist *et al*. 2011a). For more details on the study system see Sundqvist *et al*. (2011a,b), Fig. 1 and Table S1.

In September 2007 four replicate plots (each 10 x 10 m) were located in each of the vegetation types, at every 100 m along an elevational gradient ranging from 500 to 1000 m, yielding a total of 48 plots. The plots, centred on smaller (2 m x 2 m) plots, used in a previous study across this gradient (Sundqvist *et al*. 2011a). To ensure that pseudoreplication was minimized within each elevation, the mean distance of each plot to the next nearest plot was approximately 15 m (with the mean distance between the two most distance plots being approximately 100 m). Because of the high spatial heterogeneity over short distances (i.e., in the order of a few meters) in microtopography, hydrology and soil fertility in these communities (Björk *et al.* 2007), it is expected that the 15 m distance among plots is sufficient to ensure adequate independence among them. The plots at 500 m are situated in open birch forest, plots at 600 m are located immediately above the forest line, and plots at 700-1000 m sites are situated above the treeline.

In order to explore the effect of elevation (i.e. decline in temperature and soil fertility) on leaf litter phenolic properties, senesced fresh leaf litter was hand-collected from three to four plant species in each plot between 6 and 17 September 2008. This yielded a total of 13 plant species; eight species were collected in meadow vegetation (*Bartsia alpina*, *Carex aquatilis* ssp. *stans*, *Carex saxatilis, Geranium sylvaticum, Sibbaldia procumbens*, *S. virgaurea*, *Salix polaris* and *T. europaeus*), three species were collected in heath vegetation (*E. hermaphroditum, B. nana*, *V. vitis-idaea*), and two species were collected in both heath and meadow (*C. bigelowii, B. pubescens* ssp. *czerepanovii*). These species were selected to ensure that we included species that are common on a portion of the gradient, as well as those species that occurred across most or the entire gradient, which allows us to effectively explore variation both across and within species across the gradient (Wardle *et al.* 2009, Sundqvist *et al.* 2011b). For each species, a quantity of at least 1 g of senesced leaves still attached to the stem, representing a minimum of 30 individual leaves, were collected from each plot and all materials were air dried (>22$^{o}$C) after collection. Green leaf traits are often strongly related to litter quality (Cornwell *et al*. 2008; Fortunel *et al*. 2009), including phenolic content (Pastor & Naiman 1992; Schweitzer *et al*. 2004). Our approach thus provides a relative comparison of phenolic properties and their potential litter afterlife effects, among and within species, across our study system. For details on species functional groups and at which elevations each species was collected, see Table S2 in Supporting Information.

### *Laboratory analysis*

For each litter sample, litter extracts were created by extracting 0.3 g of ground litter (ball mill, Retsch, MM 301) in 100 mL of deionized water (DI) and left to shake for 24 hours, after which samples were filtered through coarse filters, followed by 0.2 µm disposable filters under vacuum (Garnett *et al*. 2004; Joanisse, Bradley & Preston 2008). We extracted with deionized water because water soluble phenolics represent the most biologically active fraction of the total pool of phenolics, and previous work using many of the same species has shown that phenolic concentrations in water extracts are strongly correlated with phenolic concentrations in organic solvent extracts (Gundale *et al*. 2010). The Prussian blue technique (Stern *et al*. 1996) and acid-butanol method (Porter, Hrstich & Chan 1986) were used to measure total extractable phenolics and total amount of condensed tannins within these extracts, using catechin (+/-) and procyanidin B2 (Sigma-Aldrich) as standards, respectively. The total concentrations of phenolics and tannins are reported on a per gram of litter dry weight basis.

The protein complexation capacity (PCC) of each litter extract was quantified using the method described by Gundale *et al*. (2010), whereby an external protein (Bovine Serum Albimun; BSA) is added to each litter extract and the quantity of precipitated protein is measured. The PCC is reported on a per gram of litter dry weight basis. Further details of the method are given by Gundale *et al*. (2010). This PCC method provides an estimate of protein complexation by all tannins present in the extract, including condensed tannins and the less easily quantifiable hydrolizable fraction. Therefore, for each sample we used the data for total PCC and total phenolics to estimate the amount of protein complexed per unit of total phenol.

### *Statistical analysis*

To determine whether each phenolic response variable (i.e. total phenolics, tannins, PCC, and PCC per unit phenol) within individual species responded to the elevational gradient, we used one-way ANOVA for each of five species that occurred on at least four of the six elevation sites within the gradient, i.e., *B. nana*, *E. hermaphroditum*, *V. vitis-idaea*, *B. alpina* and *T. europaeus* (Table S2). Whenever significant effects of elevation were found within species, differences among means were explored using Tukey's honestly significant difference (h.s.d.) at $p = 0.05$. To analyse variation in each phenolic response variable at the across-species level, we calculated the mean value of each response variable for each species across all elevations, and the mean elevation at which each species occurred. We recognize that some species may potentially occur above the highest elevation or below the lowest elevation used in our study, but emphasize that these mean elevation values for each species are intended only for comparative purposes, i.e., comparison among species within the range of elevations that we considered. We then used linear regression on these calculated mean values to test for the relationship of each response variable with elevation across this elevational range, with each species serving as an independent data point, as described by Wardle *et al*. (2009) and Sundqvist *et al*. (2011b). To assess the effect of vegetation type and elevation on each response variable at the community-level, we used a community weighted average approach (Garnier *et al*. 2007). All species within a given plot were weighted according to their relative abundance in that plot, based on previously published species abundance data (Sundqvist *et al*. 2011a), yielding a single mean value of each variable for each plot (Garnier *et al*. 2007; Fortunel *et al*. 2009). For each variable, the weighted average value for each plot was calculated by the following equation according to Garnier *et al*. (2007):

$$\text{variable}_{aggr} = \sum_{i=1}^{n} p_i \times \text{variable}_i$$

where $\text{variable}_{aggr}$ is the aggregated value of that variable for all species collected in that plot, $p_i$ is the cover of species *i* as a proportion of the total cover for all species collected in that plot, and $\text{variable}_i$ is the value for that variable for species *i*. For each plot and for each variable we also calculated the average of all species for each plot without weighting for relative abundance, as used by Wardle et al. (2009) and Mason et al. (2012), to determine a plot average value. We used two-way ANOVA to test for the effects of vegetation type and elevation (and their interaction) on $\text{variable}_{aggr}$ (i.e. the plot weighted average values) and the plot average value for each response variable. When ANOVA yielded significant treatment effects of elevation, differences among means were further analysed using Tukey's h.s.d. at $p = 0.05$. Using the plot average values and $\text{variable}_{aggr}$ yielded similar results and we therefore report our results for $\text{variable}_{aggr}$. For all data analysis, all data variables were log-transformed when required, to comply with the assumptions of parametric tests. All statistical analyses were performed using SPSS (PASW statistics 18.0).

## Results

### *Effect of elevation within- and across species*

At the within-species level, elevation (and thus declining temperature and soil fertility) had significant effect on total phenolic content for three of the five species we studied, i.e., two from the heath (*E. hermaphroditum, V. vitis-idaea*) and one from the meadow (*T. europaeus*) (Fig. 2a). Total phenolic content for *E. hermaphroditum* was highest at the 600 m elevation and lowest at 900 m, while that for *V. vitis-idaea* was lowest at the 500 m elevation and greatest at the three highest elevations. For *T. europaeus*, the total phenolic content was highest at 600 m and lowest at intermediate elevations. Further, elevation also had a significant effect on the total tannin content, the PCC and the PCC to phenol ratio for *E. hermaphroditum* and *V. vitis-idaea* (Fig. 2b-d). For *E. hermaphroditum*, total tannin content and PCC was significantly higher at the lowest elevation than at the third and second highest elevations, respectively, while the amount of protein complexed per unit phenol was significantly higher at the lowest elevation than at the intermediate and highest elevations. For *V. vitis-idaea*, the total tannin content was highest at 900 m and lowest at 500 and 700 m, and the PCC and the amount of protein complexed per unit phenol were both highest at the lowest elevation (Fig. 2d). No response variables for the other two species (*B. nana* and *B. alpina*) showed any relationship with elevation (Fig. 2).

At the across-species level (when each species for each vegetation type was represented as a single data point) there was a significant negative relationship between total phenolics and elevation across the elevational range that we considered, while PCC was marginally nonsignificantly negatively related to elevation at $p = 0.05$ (Fig. 3). Total tannins and the PCC to phenol ratio had no relationship with elevation ($R^2 = 0.011$, $p = 0.711$; and $R^2 = 0.002$, $p = 0.871$; respectively).

### *Effect of vegetation type and elevation at the whole plot level*

When community weighted measures were used to assess each response variable at the whole plot level, there was a significant main effect of vegetation type on the total tannin content in leaf litter and the amount of protein complexed per unit phenol in leaf litter (Table 1). Overall the total tannin content was significantly higher for the heath than for the meadow while the meadow had a higher amount of protein complexed per unit phenol than did the heath (Fig. 4b,d). Further, there was a significant main effect of elevation and interactive effect of elevation and vegetation on all four variables measured (Table 1). For the heath, the total phenolic concentration was significantly higher at 800 m than at 900 m and no other elevations differed significantly from each other. For the meadow, the phenolic concentration was significantly higher at 500 m and 700 m than at the three highest elevations (Fig. 4a). Elevation had no effect on total tannin content for the heath but for the meadow tannins were significantly higher at the highest elevation compared to 600 m and 700-800 m elevations (Fig. 4b). The PCC was significantly higher at the lowest elevation relative to all other elevations for the heath, and relative to all elevations except at 600 m for the meadow (Fig. 4c). Further, the amount of protein complexed per unit phenol was significantly higher at the lowest elevation relative to all other elevations for the heath, and relative to the 700 m and 900 m elevations for the meadow (Fig. 4d).

## Discussion

### *Within- and across species variation in litter phenolic properties*

Increasing environmental stress and low nutrient availability are proposed to be primary factors controlling inter- and intra-specific variation in plant phenolic levels (Bryant *et al*. 1983; Coley *et al*. 1985), and in influencing plant-soil feedbacks that result from plant litter entering the soil environment (Hättenschwiler & Vitousek 2000; Joanisse *et al*. 2009). Therefore, we hypothesized that an increase in elevation, and thus in environmental stress and nutrient limitation, would lead to increases in total phenolic and tannin concentrations and protein complexation capacity (PCC) of plant litter both within and among species. At the within-species level, only one species (*V. vitis-idaea*) supported this prediction by showing a higher content of total phenolics with increasing elevation and thus declining soil nutrient availability (Sundqvist *et al*. 2011a) and leaf nutrient concentrations (Sundqvist *et al*. 2011b). In contrast, the most dominant species in the heath community type, *E. hermaphroditum*, displayed the reverse pattern for all phenolic properties we measured, despite increasing elevation being associated with declining soil nutrient availability and leaf nutrient concentrations in *E. hermaphroditum* (Fig. 1; Sundqvist *et al*. 2011a,b). These data are not strongly supportive of theories predicting how patterns of plant phenolic content (Bryant *et al*. 1983; Coley *et al*. 1985; Close & McArthur 2002) or resultant plant-soil feedbacks (Hättenschwiler & Vitousek 2000; Joanisse *et al*. 2009) respond to stress across environmental gradients. Instead they show that there can be considerable differences among co-existing species in how their phenolic properties respond to gradients of abiotic stress and soil fertility (e.g. Koricheva *et al*. 1998; Hamilton *et al*. 2001; Hansen *et al*. 2006).

It is well known that phenolics are a diverse group of compounds that can differ greatly in the strength of their effects on specific processes, such as herbivory, litter decomposition, nutrient cycling and allelopathic effects against other plants (Schimel, Cates & Ruess 1998; Meier & Bowman 2008; Barbehenn & Constabel 2011). It is also recognized that tannins exhibit great variability in their protein complexation capacity and effect on litter decomposition (Coq *et al*. 2010; Salminen & Karonen 2011; Hättenschwiler *et al*. 2011). Consistent with this, we found for *V. vitis-idaea* that although the total phenolic and tannin contents were low at the lowest elevation, both PCC and the amount of protein complexed per unit of phenol were highest at this elevation. As such, the PCC of *V. vitis-idaea* litter across our study system was greatest at the elevation at which the overall highest leaf and litter nutrient concentrations occur for this species (Sundqvist et al. 2011b). Further, we found that among our most abundant species, only 3-5 % of the dry weight of *E. hermaphroditum* litter consisted of phenolic compounds, versus 7-14 % for *V. vitis-idaea* and 7-13 % for *B. nana* (Fig. 2). Despite the lower phenolic concentrations of *E. hermaphroditum*, previous studies have shown that one of the main phenolics produced by this species is the low molecular weight phenolic compound, batatasin-III, which has been suggested to have strong negative effects on belowground processes, and allelopathic effects on other plants, even at very low concentrations (Nilsson 1994; Wardle *et al*. 1998; Tybirk *et al*. 2000; Wallstedt, Gallet & Nilsson 2005). Our results further highlight the importance of considering not only the total quantity of phenolics that a plant produces but also the properties and composition of those phenolics (Hättenschwiler *et al*. 2011) and how they may vary among species that occur on the same environmental gradient.

In contrast to our second hypothesis, and previous findings from this system that increasing elevation is associated with a decline in SLA and leaf and litter P concentrations and an increase in N:P ratios (Sundqvist *et al*. 2011b), we found that those species that were most abundant at higher elevations generally produced litter which had the lowest concentrations of phenolics and PCC (Fig. 3). These results are further inconsistent with theories proposing a greater importance of plant phenolics in generating stronger plant-soil feedbacks in low fertility environments (Hättenschwiler & Vitousek 2000; Northup et al. 1995). They are instead consistent with studies in boreal forests showing that litter phenolics can often be higher for species that dominate on more fertile soils (Wardle *et al*. 2003; Gundale *et al*. 2010). Our results are also consistent both with studies which have found foliar phenolic content to decline with increasing elevation (Carey & Wink 1994; Wallis, Huber & Lewis 2011) and decreasing temperatures (Jonasson *et al*. 1986; Hansen *et al*. 2006). Several mechanisms have been proposed to explain such patterns (Carey & Wink 1994; Hansen *et al*. 2006; Zidorn 2010). In a subarctic tundra system, Jonasson *et al*. (1986) studied the effect of inter-annual variation in macroclimate on plant chemical properties and proposed that, within their site, increasing temperature may cause plant photosynthetic rates to increase more than plant nutrient availability. This was in turn proposed to cause a plant carbon surplus, which enables plants to allocate a greater amount of carbon to secondary chemistry production rather than to growth (Bryant *et al*. 1983). In contrast to Jonasson *et al*. (1986), in our study system both temperature and soil nutrient availability is highest at the lowest elevations for the heath (Sundqvist et al. 2011a). In addition, across all species in our study system, those that dominate at low elevations display several functional traits associated with faster growth and higher nutrient acquisition compared to those species that dominate at high elevations (Sundqvist et al. 2011b), suggesting that the mechanism proposed by Jonasson *et al.* (1986) is unlikely to explain the decline in phenolic properties with elevation we observed. Alternatively, there is increasing evidence that low temperatures at high elevations limit plant meristematic activity and synthetic process rates rather than photosynthesis (Hoch & Körner 2003; Shi, Körner, & Hoch 2008; Hoch & Körner 2009), potentially leading to the plant having a reduced capacity to synthesize complex molecules such as phenolics despite having an excess of C available for synthesis.

### *Variation in phenolic properties across plant community types and elevation*

We used community abundance-weighted measures to test our third hypothesis that, at the whole plot level, leaf litter produced by heath communities (i.e. dominated by ericaceous dwarf shrubs) and occurring on nutrient poor soils should have higher amounts of phenolic compounds than meadow communities dominated by herbaceous species and occurring on more fertile soils. Consistent with this, total amounts of tannins were highest in heath plots. This result supports previous suggestions that the protein-tannin complexes in the litters produced by some ericaceous plants can be accessed via their mycorrhizal fungal associates and thus allows them to potentially exclude other, non-ericaceous species, by reducing their access to nitrogen (Joanisse *et al*. 2009; Wurzburger & Henrick 2009). However, in our study, litter phenolic concentrations and PCC did not differ between the vegetation types and, against our expectations, the meadow community produced litter which contained phenolics that had a higher total capacity to complex proteins than did the heath. Previous studies have revealed that there may be a great variability among species in terms of the different tannins they produce (e.g. Kraus *et al*. 2003b). Our results could be due to a higher concentration of hydrolyzable tannins in the meadow species (which we were unable to quantify) and are consistent with a study by Saetnan & Batzli (2009) showing that herbaceous species dominating in more fertile sites in a Norwegian alpine ecosystem produces higher levels of total phenolics (Saetnan & Batzli 2009). Further, because species in the heath likely root and recycle nutrients from within the organic layer, while some meadow species might root and access nutrients from within the mineral soil, differences in rooting depth for heath and meadow species may also help explain these findings. In total, our results highlight that the ecological functions of specific phenolic compounds produced by different species or vegetation types (Salminen & Karonen 2011; Hättenschwiler *et al*. 2011) may be more relevant for understanding plant-soil feedbacks than total phenolic or tannin pool sizes *per se*.

It is recognized that both within species variability and species turnover are important mechanisms by which communities respond to environmental variation (Schweitzer *et al*. 2008; Lepš *et al*. 2011) but few studies have evaluated the relative importance of these factors among different plant community types. We hypothesized that the litter phenolic properties of the meadow community would be more responsive to elevation, and associated shifts in temperature and soil fertility, than those of the heath community. This hypothesis was based on the previously documented higher rate of species turnover with increasing elevation for the meadow relative to heath (Sundqvist *et al*. 2011a) which is associated with a greater responsiveness to elevation of plant leaf and litter traits, and litter decomposability (Fig. 1.; Sundqvist *et al.* 2011b). In support of this, all phenolic properties we measured showed a stronger relationship with elevation for the meadow community than for the heath, consistent with findings that the responsiveness of community functional traits is greater across abiotic gradients which have a high degree of species turnover (Wardle *et al*. 2009). Our results for the heath also show that even when individual species are highly responsive to a gradient (Fig. 2), community-level trait responses can remain very stable as long as individual species responses offset one-another. Therefore, our data highlights that the relative importance of intra-specific variation versus species turnover in determining the response of community traits to environmental gradients may differ greatly for contrasting, but co-occurring, plant community types. Shifts in community traits (i.e., litter chemical properties) in response to changes in abiotic factors can be a strong predictor of how ecosystem properties and processes (i.e. decomposition rates and nutrient availability) respond to the same abiotic factors (Suding *et al*. 2008). Our results therefore suggest that responses to elevation of ecosystem properties and processes that are driven by litter phenolics may differ considerably among plant community types.

### *Conclusions*

Our study did not provide support for our hypotheses in that we did not find increases in phenolics in plant leaf litter with increasing elevation (and thus declining temperature and nutrient availability) within species, across species, or at the whole community level. It also revealed large differences among species in how their litter phenolic properties responded to elevation, suggesting that responses of phenolics to environmental factors are highly individualistic, rather than regulated by universal mechanisms as predicted by several theories on plant phenolic allocation (e.g. Bryant *et al*. 1983; Coley *et al*. 1985). Further, and also inconsistent with such theories, total phenolics in leaf litter did not differ between the heath vegetation (dominating on nutrient poor soils) and the meadow vegetation (dominating on more fertile soils). Instead, our results highlight the importance of considering not only the total amount of phenolics in litter produced by different plant species and communities, but also the ecological functions of those phenolics (Hättenschwiler *et al*. 2011). Finally, our results showed that the responsiveness of phenolic properties to elevation was greater in meadow than in heath vegetation, indicating that large differences can exist among contrasting plant community types in how litter chemical traits at the plant community level respond to abiotic gradients. These types of differences are in turn likely to have consequences for ecosystem properties and processes driven by litter chemical traits, such as litter decomposition, nutrient cycling, plant nutrition and plant growth (Suding *et al*. 2008).

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## References

Barbehenn, R.V. & Constabel, C.P. (2011) Tannins in plant-herbivore interactions. *Phytochemistry*, **72**, 1551-1556.

Berendse, F. (1994) Litter decomposability – a neglected component of plant fitness. *Journal of Ecology*, **82**, 187-190.

Björk, R.G., Klemedtsson, L., Molau, U., Harndorf J., Ödman, A. & Giesler, R. (2007) Linkages between N turnover and plant community structure in a tundra landscape. *Plant and Soil*, **294**, 247-261.

Bryant, J.P., Chapin, F.S., III & Klein, D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357-368.

Close, D.C. & McArthur, C. (2002) Rethinking the role of many plant phenolics – protections from photodamage not herbivores? *Oikos*, **99**, 166-172.

Coley, P.D., Bryant, J.P. & Chapin, S.F., III. (1985) Resource availability and plant antiherbivore defense. *Science*, **230,** 895-899.

Cornelissen, J.H.C., Quested, H.M., Gwynn-Jones, D., Van Logtestijn, R.S.P., De Beus, M.A.H., Kondratchuk A., Callaghan T.V. & Aerts, R. (2004) Leaf digestability and litter decomposability are related in a wide range of subarctic plant species and types. *Functional Ecology*, **18**, 779-786.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T.V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V., Westoby, M. (2008) Plant species traits are the predominant control of litter decomposition rates within biomes worldwide. *Ecology Letters* **11**, 1065–1071.

Coq, S., Souquet, J.-M., Meudec, E., Cheynier, V. & Hättenschwiler, S. (2010) Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana. *Ecology*, **91**, 2080-2091.

Eskelinen, A., Stark, S. & Männistö, M. (2009) Links between plant community composition, soil organic matter quality and microbial communities in contrasting tundra habitats. *Oecologia*, **161**, 113-123.

Fortunel, C., Garnier, E., Joffre, R., Kazakou, E., Quested, H., Grigulis, K., Lavorel, S., Ansquer, P., Castro, H., Cruz, P., Doležal, J., Eriksson, O., Freitas, H., Golodets, C., Jouany, C., Kigel, J.,

Fukami, T. & Wardle, D.A. (2005) Long-term ecological dynamics: reciprocal insight from natural and anthropogenic gradients. *Proceedings of the Royal Society of London, Series B – Biological Sciences* **272**, 2105-2115.

Garnett, E., Jonsson, L.M., Dighton, J. & Murnen, K. (2004) Control of pitch pine seed germination and initial growth exerted by leaf litters and polyphenolic compounds. *Biology and Fertility of Soils*, **40**, 421-426.

Garnier, E., Lavorel, S., Ansquer, P., Castro, H., Cruz, P., Dolezal, J., Eriksson, O., Fortunel, C., Freitas, H., Golodets, C., Grigulis, K., Jouany, C., Kazakou, E., Kigel, J., Kleyer, M., Lehsten, V., Lepš, J., Meier, T., Pakeman, R., Papadimitriou, M., Papanastasis, V.P., Quested, H., Quétier, F., Robson, M., Roumet, C., Rusch, G., Skarpe, C., Sternberg, M., Theu, J-P., Thébault, A., Vile, D. & Zarovali, M.P. (2007) Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: A standardized methodology and lessons from an application to 11 European sites. *Annals of Botany*, **99**, 967-985.

Giesler, R., Esberg, C., Lagerström, A. & Graae, B.J. (2011) Phosphorus availability and microbial respiration across different tundra vegetation types. *Biogeochemistry*, DOI 10.1007/s10533-011-9609-8.

Goodall, D.W. (1952) Some considerations in the use of point quadrats for the analysis of vegetation. *Australian Journal of Scientific Research, Series B: Biological Sciences*, **5**, 1-41.

Gundale, M.J., Sverker, J., Albrectsen, B.R., Nilsson M-C. & Wardle, D.A. (2010) Variation in protein complexation capacity among and within six plant species across a boreal forest chorosequence. *Plant Ecology*, **211**, 253-266.

Hagerman, A.E. (1987) Radial diffusion method for determining tannin in plant-extracts. *Journal of Chemical Ecology*, **13**, 437-449.

Hamilton, J.G., Zangerl, A.R., DeLucia, E.H. & Berenbaum, M.R. (2001) The carbon-nutrient balance hypothesis: its rise and fall. *Ecology Letters*, **4**, 86-95.

Hansen, A.H., Jonasson, S., Michelsen, A. & Julkunen-Tiitto, R. (2006) Long-term experimental warming, shading and nutrient addition affect the concentration of phenolic compounds in arctic-alpine deciduous and evergreen dwarf shrubs. *Oecologia*, **147**, 1-11.

Hoch, G. & Körner, C. (2003) The carbon charging of pines at the climatic treeline: a global comparison. *Oecologia*, **135**, 10-21.

Hoch, G. & Körner, C. (2009) Growth and carbon relations of tree line forming conifers at constant vs. variable low temperatures. *Journal of Ecology*, **97**, 57-66.

Huston, M.A. & DeAngelis, D.L. (1994) Competition and Coexistance: The effects of resource transport and supply rates. *The American Naturalist*, **144**, 954-977.

Hättenschwiler, S., Coq, S., Barantal, S. & Handa, I.T. (2011) Leaf traits and decomposition in tropical rainforests: revisiting some commonly held views and towards a new hypothesis. *New Phytologist*, **189**, 950-965.

Hättenschwiler, S., Hagerman, A.E. & Vitousek, P.M. (2003) Polyphenols in litter from tropical montane forests across a wide range in soil fertility. *Biogeochemistry*, **64**, 129-148.

Hättenschwiler, S. & Vitousek, P.M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in ecology and evolution*, **15**, 238-234

Joanisse, G.D., Bradley, R.L. & Preston, C.M. (2008) Do late-successional tannin-rich plant communities occurring on highly acidic soils increase the DON/DIN ratio? *Biology and Fertility of Soils*, **44**, 903-907.

Joanisse, G.D., Bradley, R.L., Preston, C.M. & Bending, G.D. (2009) Sequestration of soil nitrogen as tannin-protein complexes may improve the competitive ability of sheep laurel (*Kalmia angustifolia*) relative to black spruce (*Picea marina*). *New Phytologist*, **181**, 187-198.

Joanisse, G.D., Bradley, R.L., Preston, C.M. & Munson, A.D. (2007) Soil enzyme inhibition by condensed litter tannins may drive ecosystem structure and processes: the case of *Kalmia angustifolia*. *New Phytologist*, **175**, 535-546.

Jonasson, S., Bryant, J.P., Chapin, F.S. III, & Andersson, M. (1986) Plant phenols and nutrients in relation to variations in climate and rodent grazing. *The American Naturalist*, **128**. 394-408.

Jones, C.G. & Hartley, S.E. (1999) A protein competition model of phenolic allocation. *Oikos*, **86**, 27-44.

Koricheva, J., Larsson, S., Haukioja, E. & Keinanen, M. (1998) Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos*, **83**, 212-226.

Koricheva, J. (2002) The Carbon-Nutrient Balance Hypothesis is dead; long live the carbon-nutrient balance hypothesis? *Oikos*, **98**, 537-539.

Kraus, T.E.C., Dahlgren, R.A. & Zasoski, R.J. (2003a) Tannins in nutrient dynamics of forest ecosystems - a review. *Plant and Soil*, **256**, 41-66.

Kraus, T.E.C. Yu, Z, Preston, C.M., Dahlgren, R.A. & Zasoski, R.J. (2003b) Linking chemical reactivity and protein precipitation to structural characteristics of foliar tannins. *Journal of Chemical Ecology*, **29, 703-730.**

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology and Evolution*, **22**, 569-574.

Lepš, J., de Bello, F., Šmilauer, P. & Doležal, J. (2011) Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. *Ecography*, **34**, 856-863.

Lerdau, M. & Coley, P.D. (2002) Benefits of the Carbon-Nutrient Balance Hypothesis. *Oikos*, **98**, 534-536.

Mason, N., Richardson, S., Peltzer, D., de Bello, F., Wardle, D. A. & Allen, R. (2012) Changes in co-existence mechanisms along a long-term soil chronosequence revealed by functional trait diversity. *Journal of Ecology*, **100,** 678-689.

Meier, C.L. & Bowman, W.D. (2008) Phenolic-rich leaf carbon fractions differentially influence microbial respiration and plant growth. *Oecologia*, **158**, 95-107.

Nilsson, M-C. (1994) Separation of allelopathy and resource competition by the boreal dwarf-shrub *Empetrum hermaphroditum* Hagerup. *Oecologia*, **98**, 1-7.

Northup, R.R., Yu, Z., Dahlgren, R.A. & Vogt, K.A. (1995) Polyphenol control of nitrogen release from pine litter. *Nature*, **377**, 227-229.

Northup, R.R., Dahlgren, R.A. & McColl, J.G. (1998) Polyphenols as regulators of plant-litter-soil interactions in northern California's pygmy forest: A positive feedback? *Biogeochemistry* **42,** 189-220.

Pastor, J. & Naiman, R.J. (1992) Selective foraging and ecosystem processes in boreal forests. *The American Naturalist*, **139**, 690-705.

Porter, L.J., Hrstich, L.N. & Chan, B.G. (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, **25**, 223-230.

Ruess, L., Michelsen, A., Schmidt, I.K. & Jonasson, S. (1999) Simulated climate change affecting microorganisms, nematode density and biodiversity in subarctic soils. *Plant and Soil*, **212**, 63-73.

Saetnan, E.R. & Batzli, G.O. (2009) Effects of simulated herbivory on defensive compounds in forage plants of Norwegian alpine rangelands. *Journal of Chemical Ecology*, **35**, 469-475.

Salminen, J-P. & Karonen, M. (2011) Chemical ecology of tannins and other phenolics: we need a change in approach. *Functional Ecology*, **25**, 325-338.

Schimel, J.P., Cates, R.G. & Ruess, R. (1998) The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. *Biogeochemistry*, **42**, 221-234.

Schweitzer, J.A., Bailey, J.K., Rehill, B.J., Martinsen, G.D., Hart, S.C., Lindroth, R.L., Keim, P. & Whitham, T.G. (2004) Genetically based trait in a dominant tree affects ecosystem processes. *Ecology Letters*, **7**, 127-134.

Schweitzer, J.A., Madritch, M.D., Bailey, J.K., LeRoy, C.J., Fischer, D.G., Rehill, B.J., Lindroth, R.L., Hagerman, A.E., Wooley, S.C., Hart, S.C. & Whitham, T.G. (2008) From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. *Ecosystems*, **11**, 1005-1020.

Shi, P., Körner, C. & Hoch, G. (2008) A test of the growth-limitation theory for alpine tree line formation in evergreen and deciduous taxa of the eastern Himalayas. *Functional Ecology*, **22**, 213-220.

Stamp, N. (2003) Out of the quagmire of plant defense hypotheses. *The Quarterly Review of Biology*, **78**, 23-55.

Stern, J.L., Hagerman, A.E., Steinberg, P.D., Winter, F.C. & Estes, J.A. (1996) A new essay for quantifying brown algal phlorotannins and comparisons to previous methods. *Journal of Chemical Ecology*, **22**, 1273-1293.

Suding, K.N., Lavorel, F.S., Chapin, F.S. III., Cornelissen, H.C., Díaz, S., Garnier, E., Goldberg, D., Hooper, D.U., Jackson, S.T. & Navas, M.-L. (2008) Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology*, **14**, 1125-1140.

Sundqvist, M.K., Giesler, R., Graae, B.J., Wallander, H., Fogelberg, E. & Wardle, D.A. (2011a) Interactive effects of vegetation type and elevation on aboveground and belowground properties in a subarctic tundra. *Oikos*, **120**, 128-142.

Sundqvist, M.K., Giesler, R. & Wardle, D.A. (2011b) Within- and across-species responses of plant traits and litter decomposition to elevation across contrasting vegetation types in subarctic tundra. PLoS ONE 6(10): e27056. doi:10.1371/journal.pone.0027056

Tilman, D. (1985) The resource-ratio hypothesis of plant succession. *The American Naturalist,* **125**, 827-852.

Tybirk, K., Nilsson, M-C., Michelsen, A., Lakkenborg Kristensen, H., Schevtsova, A., Strandberg, M.T., Johansson, M., Nielsen, K.E., Riis-Nielsen, T., Strandberg, B. & Johnsen, I. (2000) Nordic Empetrum dominated ecosystems: Function and susceptibility to climate change. *AMBIO*, **29**, 90-97.

Wallis, C.M., Huber, D.P.W. & Lewis, K.J. (2011) Ecosystem, location, and climate effects on foliar secondary metabolites of Lodgepole pine populations from central British Colombia. *Journal of Chemical Ecology*, **37**, 607-621.

Wallstedt, A., Gallet, C. & Nilsson, M-C. (2005) Behaviour and recovery of the secondary metabolite batatasin-III from boreal forest humus: influence of temperature, humus type and microbial community. *Biochemical Systematics and Ecology*, **33**, 385-407.

Wardle, D.A., Bardgett, R.D., Walker, L.R. & Bonner, K.I. (2009) Among- and within-species variation in plant litter decomposition in contrasting long-term chronosequences. *Functional Ecology*, **23**, 442-543.

Wardle, D.A., Nilsson, M-C., Gallet, C. & Zackrisson, O. (1998) An ecosystem-level perspective of allelopathy. *Biological Reviews*, **73**, 305-319.

Wardle, D.A., Nilsson, M-C., Zackrisson, O. & Gallet, C. (2003). Determinants of litter mixing effects in a Swedish boreal forest. *Soil Biology and Biochemistry*, **35**, 827-835.

Wurzburger, N., & Henrick, P.L. (2009) Plant litter chemistry and mycorrhizal roots promote a nitrogen feedback in a temperate forest. *Journal of Ecology*, **97**, 528-536.

Zidorn, C. (2010) Altitudinal variation of secondary metabolites in flowering heads of the Asteraceae: trends and causes. *Phytochemistry Reivews*, **9**, 197-203.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

**Figure S1** Daily mean air temperature (°C) during the growing season across the study site

**Table S1** Plant community characteristics and soil abiotic factors across the study site derived from Sundqvist et al. (2011a,b)

**Table S2** Species sampled for this study

Table 1. Results from a two-way ANOVA (*F* values, with *p* in parentheses) testing for the effect of vegetation type (heath versus meadow) and elevation on total phenolic content, total tannin content, protein complexation capacity (mg $g^{-1}$ litter) and the amount of protein complexed per unit phenol in plant litter. Data are weighted averages, where all species collected in each plot are weighted according to their relative abundance to provide a single value for each plot.

| | ANOVA results | | |
|---|---|---|---|
| | Vegetation type | Elevation | Vegetation × Elevation |
| Total phenolics | 0.3 (0.579) | **17.4 (<0.001)** | **24.2 (<0.001)** |
| Total tannins | **98.3 (<0.001)** | **3.4 (0.014)** | **4.5 (0.003)** |
| Protein complexation capacity[a] | 0.1 (0.801) | **25.8 (<0.001)** | **5.6 (<0.001)** |
| Protein:phenol ratio[a] | **20.4 (<0.001)** | **17.9 (<0.001)** | **3.2 (0.019)** |

Degrees of freedom for F-values are 1,33 for V; 5, 33 for E; and 5,33 for V×E. Values in boldface indicate statistical significance at $p \leq 0.05$. [a]Data were log-transformed prior analysis

Fig. 1. Summary of previous findings of responses of green leaf traits, litter decomposability and soil properties for heath and meadow communities to elevation across the study system, derived from data presented by Sundqvist et al. (2011a,b). All shapes represent trends in the data and not actual values. For each row (i.e., for each property or process), when overall differences in means between heath and meadow are statistically significant at $P = 0.05$ the shapes have different shadings (i.e., black and grey), where the overall mean for the black-shaded shape is higher than that for the grey-shaded shape. Within each vegetation type, rectangular shapes represent no response to elevation; all other shapes represent a significant responsiveness to elevation for that variable with highest values found where the shape is thickest.

Fig. 2. The mean (±SE) total phenolic content (a), total tannin content (b), protein precipitation capacity (c) and amount of protein complexed per unit phenol (d) in litter for each of five plant species across an elevational gradient, three from heath (*B. nana*, *E. hermaphroditum* and *V. vitis-idaea*) and two from meadow (*B. alpina* and *T. europaeus*). For each response variable for each species, $F$ and $p$-values (with df) are from a one-way ANOVA testing for the effect of elevation. Within each panel bars topped by the same letter are not significantly different at $p < 0.05$ (Tukey's h.s.d.).

Fig. 3. Relationship between total phenolic content (a) and protein complexation capacity (b) and elevation for 13 species from heath and meadow vegetation across the elevational range that we considered (i.e., 500 - 1000 m), where each species represents an independent data point. Each data point is the mean value of total phenolic content or protein complexation capacity for one species across all elevations, and the mean elevation at which that species occurs. Ba –

*Bartsia alpina*; Bn – *Betula nana*; Bp – *Betula pubescens* ssp. *czerepanovii*; Ca – *Carex aquatilis* ssp. *stans*; Cb – *C. bigelowii*; Cs – *C. saxatilis*; Eh – *Empetrum hermaphroditum*; Gs – *Geranium sylvaticum*; So – *Salix polaris*; Sr – *Sibbaldia procumbens*; Sv – *Solidago virgaurea*; Te – *Trollius europaeus*; Vv – *Vaccinium vitis-idaea*.

Fig. 4. Total plot-level mean (±SE) phenolic content, total tannin content, protein complexation capacity, and protein complexed per unit phenol for heath and meadow vegetation across an elevational gradient. All species collected in each plot are weighted according to their relative abundance to provide a single value for each plot. Within each panel, bars topped by the same letter are not significantly different at $p < 0.05$ (Tukey's h.s.d) following two-way ANOVA (results in Table 1).

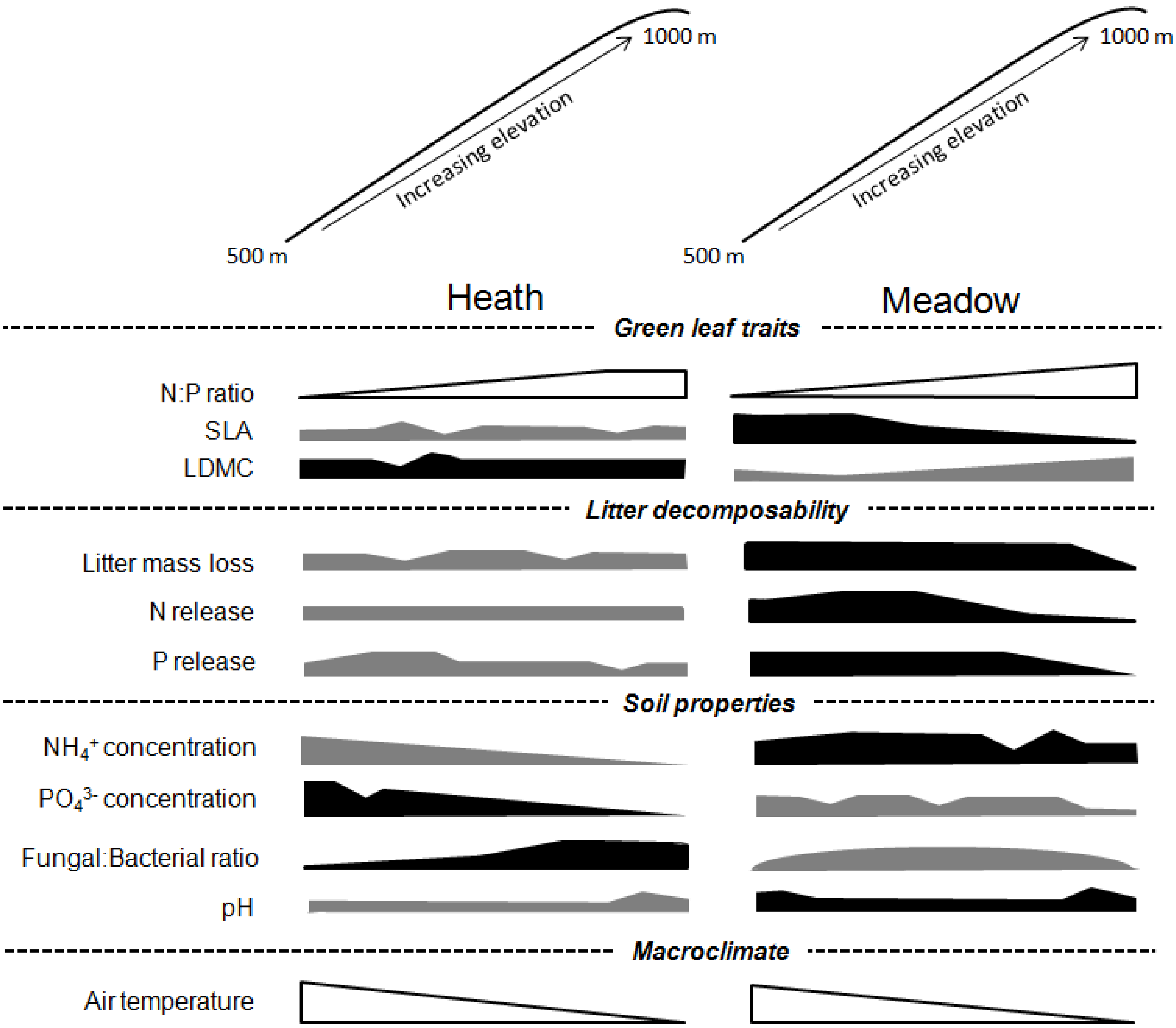

1000 m
Increasing elevation
500 m
1000 m
Increasing elevation
500 m
Heath
Meadow
Green leaf traits
N:P ratio
SLA
LDMC
Litter decomposability
Litter mass loss
N release
P release
Soil properties
$NH_4^+$ concentration
$PO_4^{3-}$ concentration
Fungal:Bacterial ratio
pH
Macroclimate
Air temperature

Figure 1

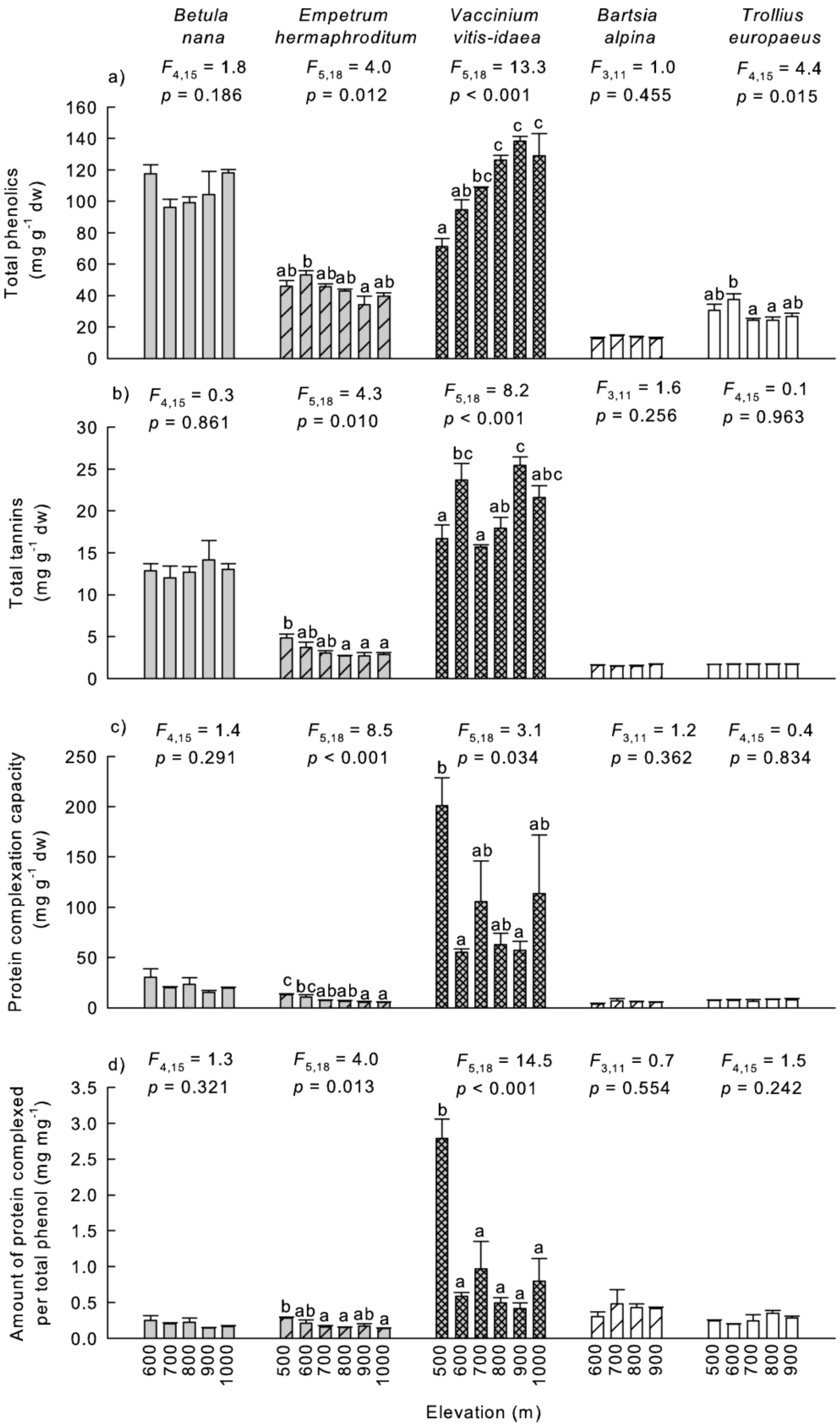

Betula nana
Empetrum hermaphroditum
Vaccinium vitis-idaea
Bartsia alpina
Trollius europaeus
a)
F4,15 = 1.8 p = 0.186
F5,18 = 4.0 p = 0.012
F5,18 = 13.3 p < 0.001
F3,11 = 1.0 p = 0.455
F4,15 = 4.4 p = 0.015
Total phenolics (mg g-1 dw)
0 20 40 60 80 100 120 140 160
ab b ab ab a ab
a ab bc c c c
ab b a a ab
b)
F4,15 = 0.3 p = 0.861
F5,18 = 4.3 p = 0.010
F5,18 = 8.2 p < 0.001
F3,11 = 1.6 p = 0.256
F4,15 = 0.1 p = 0.963
Total tannins (mg g-1 dw)
0 5 10 15 20 25 30
b ab ab a a a
a bc a ab c abc
c)
F4,15 = 1.4 p = 0.291
F5,18 = 8.5 p < 0.001
F5,18 = 3.1 p = 0.034
F3,11 = 1.2 p = 0.362
F4,15 = 0.4 p = 0.834
Protein complexation capacity (mg g-1 dw)
0 50 100 150 200 250
c bc ab ab a a
b a ab ab a ab
d)
F4,15 = 1.3 p = 0.321
F5,18 = 4.0 p = 0.013
F5,18 = 14.5 p < 0.001
F3,11 = 0.7 p = 0.554
F4,15 = 1.5 p = 0.242
Amount of protein complexed per total phenol (mg mg-1)
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
b ab a a ab a
b a a a a a
600 700 800 900 1000
500 600 700 800 900 1000
500 600 700 800 900 1000
600 700 800 900
500 600 700 800 900
Elevation (m)

Figure 2

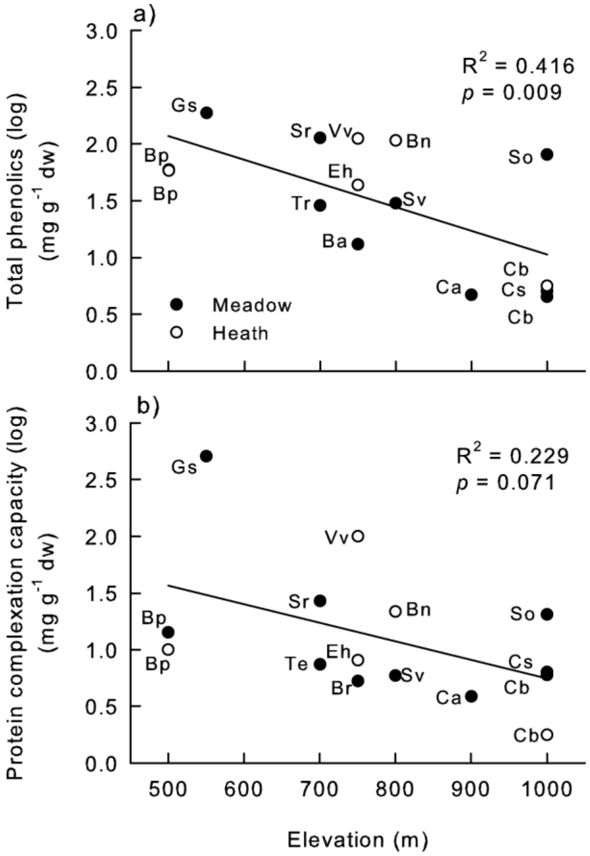
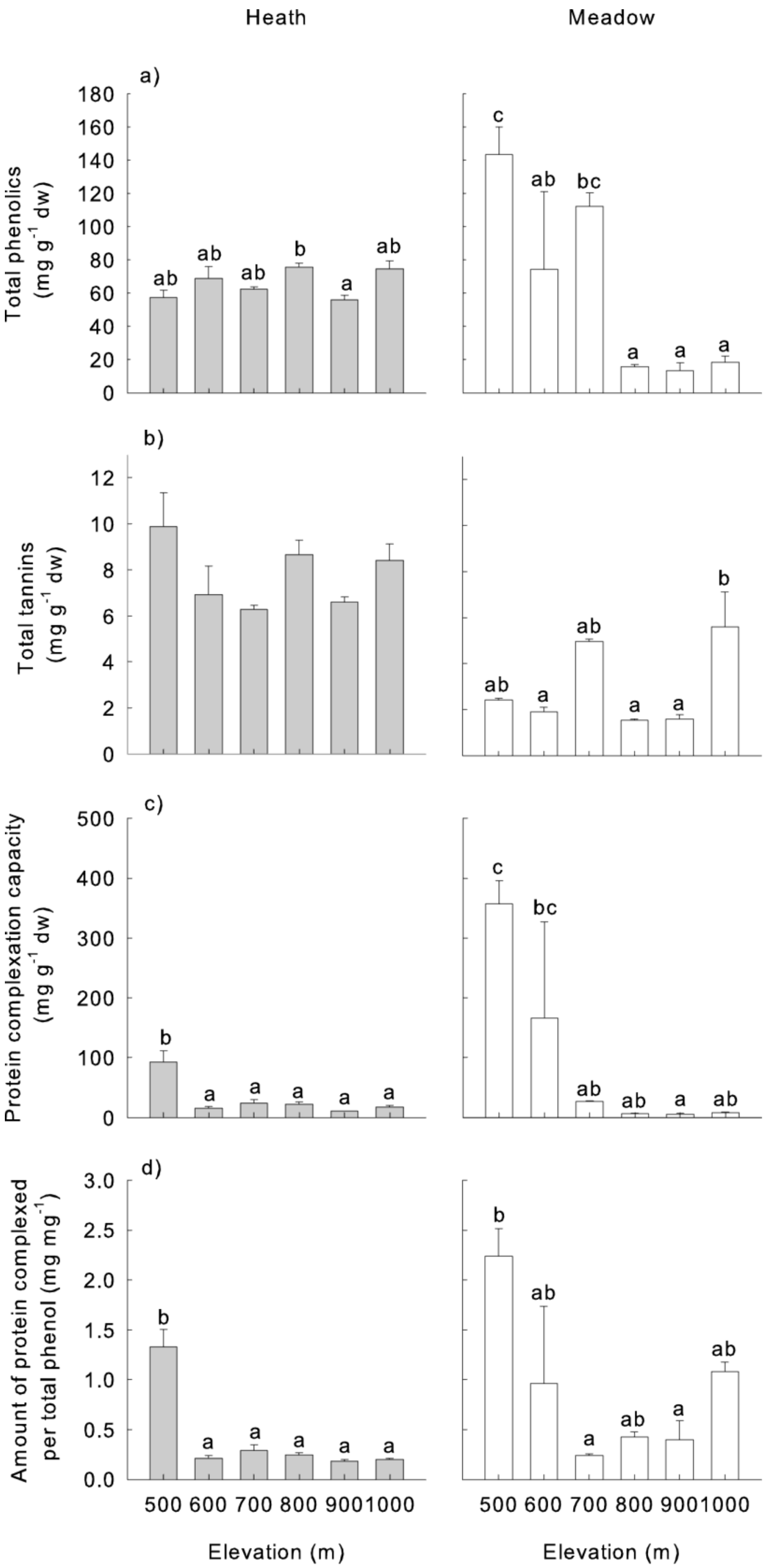

a)
Total phenolics (log) (mg g$^{-1}$ dw)
$R^2$ = 0.416
$p$ = 0.009
Gs
Sr
Vv
Bn
Bp
Bp
Eh
Tr
Sv
So
Ba
Cb
Ca
Cs
Cb
Meadow
Heath
b)
Protein complexation capacity (log) (mg g$^{-1}$ dw)
$R^2$ = 0.229
$p$ = 0.071
Gs
Vv
Sr
Bn
So
Bp
Bp
Eh
Te
Sv
Br
Ca
Cs
Cb
Cb
0.0
0.5
1.0
1.5
2.0
2.5
3.0
500
600
700
800
900
1000
Elevation (m)

Figure 3



Heath
Meadow
a)
Total phenolics (mg g$^{-1}$ dw)
180
160
140
120
100
80
60
40
20
0
ab
ab
ab
b
a
ab
c
ab
bc
a
a
a
b)
Total tannins (mg g$^{-1}$ dw)
12
10
8
6
4
2
0
ab
a
ab
a
a
b
c)
Protein complexation capacity (mg g$^{-1}$ dw)
500
400
300
200
100
0
b
a
a
a
a
a
c
bc
ab
ab
a
ab
d)
Amount of protein complexed per total phenol (mg mg$^{-1}$)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
b
a
a
a
a
a
b
ab
a
ab
a
ab
500 600 700 800 900 1000
500 600 700 800 900 1000
Elevation (m)
Elevation (m)

Figure 4