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Plant community composition and an insect outbreak influence phenol oxidase activity and soil-litter biochemistry in a sub-Arctic birch-heath.

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Abstract

Rates of decomposition in arctic soils are regulated by temperature and moisture but substrate availability is dictated by vegetation inputs, which are also subject to biotic influences. Here we examine how leaf and litter inputs from individual dwarf shrub species influence soil enzyme activity in a sub-arctic heath community in Abisko, Sweden. We further consider how foliar damage via insect herbivory (and outbreak), affect the soil community and decomposition. During the peak growing season (July 2011) we assessed how shrub community composition (*Empetrum hermaphroditum*, *Vaccinium myrtillus, V. uliginosum* and *V. vitis-idaea*) determined litter and soil phenol oxidase activity. A periodic severe outbreak of Autumn moth larvae (*Epirrita autumnata*) affected this community in the following year (July 2012) and we used this to investigate its impact on relationships with phenol oxidase activity, soil respiration, soluble NH4+ and soluble phenolics; the soluble factors being directly associated with inputs from insect larval waste (frass). Pre-outbreak (2011), the strongest relationship observed was higher phenol oxidase activity with *E. hermaphroditum* cover. In the outbreak year (2012), phenol oxidase activity had the strongest relationship with damage to the deciduous species *V. myrtillus*, with greater herbivory lowering activity. For the other deciduous species, *V. uliginosum*, soil NH4+ and phenolics were negatively correlated with foliar larval damage. Phenol oxidase activity was not affected by herbivory of the evergreen species but there was a strong positive relationship observed between *E. hermaphroditum* community abundance and soil respiration. We highlight the dominant role of *E. hermaphroditum* in such sub-Arctic shrub communities and show that even during insect outbreaks it can dictate soil processes.

Keywords: Arctic soil carbon, insect herbivory, *Empetrum*, *Vaccinium*, phenol-oxidase, plant competition

Introduction

Biophysical constraints on organic matter decomposition have underpinned globally significant accumulations of carbon (C), particularly in the organic layer of soils in northern high latitude regions (Hobbie et al. 2000; Mack et al. 2004). Warmer conditions threaten these stocks and could positively feedback on climate change via respired losses of C from Arctic soils and litter (Dorrepaal et al. 2009). Plant communities of boreal-tundra regions have characteristically low levels of primary productivity, slow rates of organic matter breakdown and low rates of nutrient turnover (Flanagan and Veum 1974; Shaver and Chapin 1980; Hobbie et al. 2002), traits that contribute to Arctic-boreal soil C storage. These communities are also sensitive to environmental change, via an ecosystem 'cascade' effect that can develop following disturbance (Post et al. 2009; Wookey et al. 2009). Perturbed soil and litter decomposition processes are the potential consequence of such cascades. Increased temperatures in the Arctic are likely to alter plant community composition, with a strong influence on soil C dynamics (Bardgett et al. 2013) and below-ground activity via root-mycorrhizal exudate quality (Bardgett et al. 2005). Microbial-rhizosphere relationships unique to each plant species (Zak and Kling 2006; van Aarle and Plassard 2010), may influence below-ground functioning on a patch scale in mixed plant communities. Few studies at high latitudes have examined how decomposition activity is determined by plant community composition and specifically how extracellular soil enzyme activity could be further modified by biotic disturbance.

Persistent episodic outbreaks of herbivory from Geometrid moth larvae affects vegetation in Arctic boreal-tundra regions and is likely to impact plant-soil dynamics, yet few studies have examined this. Work on temperate oak forest mesocosms, however, has demonstrated

increased N and C cycling following larval herbivory (Frost and Hunter 2004) Severe outbreaks of moth larvae recur periodically in spring, typically following milder winter conditions which boost egg viability and increase larval numbers (Tenow 1972). Such episodes are, hence, expected to become more frequent as the Arctic warms (Hagen et al. 2007, Jepsen et al. 2008). Such herbivore episodes could short-circuit normal C cycling processes via mobile nutrients introduced from dead plant material and animal waste, and because in general, herbivory has been shown to impact root activity as plants recover (Bardgett and Wardle 2003, Metcalfe et al. 2014).

In this study we exploited extracellular digestive enzyme activities (EEA) produced by microbial organisms and plant roots in litters and soils, using these as (bio)indicators of litter and soil decomposition dynamics over two growing seasons. Biotic decomposition processes and microbial activity are closely coupled to EEA rates (Sinsabaugh et al. 2008) and enzyme activity assays have been used widely as bio-indicators of ecological- and soil biological function (Caldwell, 2005, Kardol et al. 2010). In Arctic heaths and tundra, decomposing material contains high levels of phenolic compounds (Hansen et al. 2006). The extracellular digestive enzyme phenol oxidase may be essential for decomposition in this region because it allows access to nutrients bound in the lignin, proanthocyanidins and secondary polyphenolic compounds in this organic matter (Stafford 1988; Hattenschwiler and Vitousek 2000; Sinsabaugh 2010). Phenol oxidase enzymes (usually referred to singularly as ‘phenol oxidase’) have been identified as the key decomposer enzyme group for common peat-rich soils of northern latitudes, because phenol oxidase enables a key biochemical step (poly-phenolic oxidation) that allows organic matter breakdown in this environment (Freeman et al. 2001). Plant community composition has been linked to soil phenol oxidase activity in a temperate plant community (Kardol et al. 2010) and in principle similar drivers

may modulate phenol oxidase activity in Arctic plant communities. Generally, such relationships have not been widely tested in Arctic ecosystems, apart from a study by Zak and Kling (2006) who examined variations in phenol oxidase activity at the community-level, together with a suite of extracellular enzymes, across a range of tundra communities.

To enable this assessment, we measured phenol oxidase activity, soil respiration rates and mobile (water soluble) litter chemistry in a plant community with four co-existing ericaceous shrub species. Our aims were firstly, to investigate the influence of plant species cover on phenol oxidase activity in this plant community (according to relative spatial differences in plant community composition), and secondly, to determine whether an anticipated moth larval outbreak (in 2012) affected phenol oxidase activity, and related parameters of litter biochemistry and C dynamics. We also sought to determine if responses to the moth outbreak varied in patches dominated by different species in this community. We firstly hypothesised that phenol-oxidase activity would be modulated by plant community composition, because spatial differences in litter type and root activity would occur with transitions in species abundance (at a patch-scale). The moth larval outbreak in the preceding year was expected to reduce phenol oxidase activity because frass (insect waste) may return mobile phenolics and nitrogen compounds to soil (Barbehenn and Martin 1992; Frost and Hunter 2004), and nitrogen enrichment of highly lignified organic material is considered to inhibit the production of phenol oxidase and result in highly-recalcitrant compounds that resist decomposition (Hobbie 2008). Additionally, any precipitation washed through the damaged vegetation canopy would input further nutrients and phenolics from exposed foliar tissues (Tukey & Morgan 1963). In combination, therefore, these biochemical inputs from herbivory were expected to drive negative relationships between larval herbivory and phenol oxidase activity, due to aforementioned impacts from nitrogen (via increased levels of mobile

NH_4^+ in litter) and the influence of higher molecular weight phenolics in litter, including condensed tannins, that may inhibit phenol oxidase activity (Allison, 2006). By contrast, soil respiration rates were expected to be increased by herbivory, given an expected positive effect of enriched biochemical inputs reaching the decomposer microbial community, resulting in greater microbial activity.

Materials and methods

Experimental site and location

We sampled litter and soils from a 25 x 25m designated experimental study area of sub-Arctic birch heath, 60m due north of the Abisko Research Station in northern Sweden (68.35 °N, 18.82°E) (Fig. 1). The study area lies at the interface between Arctic-boreal and tundra biomes. The vegetation is sub-arctic *Empetrum-Vaccinium myrtillus* dwarf shrub heath, as described by Sonesson and Lundberg (1974) and Oksanen and Virtanen (1995), comprising a mosaic of ericaceous shrub vegetation largely dominated by *Empetrum hermaphroditum* L. (quadrat cover range 0-61%, mean cover 18% ± 3.5), with discrete highly abundant patches of *Vaccinium myrtillus* L. (0-44%, 6.9% ± 2.1), *V. vitis-idaea* L. (0-62%, 11.2% ± 2.9), or *V. uliginosum* L. (0-55%, 7.9 ± 2.5) (figures in parentheses are taken from our 2011 survey of the experimental area). The soils in the area comprise a 2-20cm deep layer of highly organic material ('O-horizon'), overlying glacial till interspersed by occasional erratics. This shallow soil at the experimental site thaws completely to the mineral layer during summer and maintains a stable temperature of ~7^{o}C during July-August (*pers. obs*. A. Jones). Mean monthly precipitation levels during July-August are 59.2 mm. Our investigations were conducted during two summer growing seasons (July 2011 and July 2012). Extracellular enzyme activities can vary greatly with season, being most inconsistent around the spring thaw (Jefferies et al. 2010), hence, the detectability of enzymatic signals (e.g. from long-term

climate change drivers) in spring may be unfeasible due to sudden nutrient release from thawing soils and litter (Henry 2012). By contrast, litter activities in frozen winter soils or litters are also difficult to measure. Oxidising enzyme activities in Arctic soils have been found to peak during summer (June-July) and decline during the late-summer early-autumn senescence period (August-September) (Wallenstein et al. 2009, Sistla & Schimel 2013). We chose to sample in summer (July) because phenol oxidase activities would be consistent and active, and therefore functionally testable against our chosen (biotic) drivers.

In 2011, we investigated the influence of plant species cover on soil and litter phenol oxidase activity. In 2012, a naturally recurring episodic outbreak of autumnal moth larvae (*Epirrita autumnata* Borkhausen) was used to investigate how herbivory modulated litter phenol oxidase activity, mobile litter NH_4^+ and phenolics, and soil respiration rates, as driven by differences in plant community composition.

Plant community composition and soil phenol oxidase activity

Samples for the species composition study (22 July 2011) were taken using a micro corer (10mm diameter) and a composite approach, whereby multiple sub-samples were combined and homogenised for each sample point. Soil and litter samples were obtained from the 25 x 25m experimental area, at the intersections of regular grid co-ordinates. The experimental vegetation area used had a flat aspect, low density birch canopy and a continuous understorey shrub community. Hence, it was relatively homogeneous, apart from differences in shrub community abundance at a patch scale. The shrub understorey in this habitat is a species mosaic where discrete patches (~ 0.1-0.5 m^2) of the four main species dominate and between these, a continuum of relative changes in community abundance is evident. Sampling in the 2011 assessment took place at 32 grid intersections (soil and litter) using 5m grid spacing (for

further information on the sampling grid arrangement see Online Resource 1). At each sampling point, a 16 x 16 cm grid quadrat frame defined the collection area for litter and/or soil. A 10 mm diameter corer (for soils) was used to obtain samples at 0-5 cm depth (extracting approximately ~100g of material from 15 sub-sample cores, which were combined and homogenised for each sampling point). Samples from the matrix of litter suspended between the shrub canopy and the soil (i.e. 'shrub understorey' litter) were hand collected. This litter layer is characteristic of many undisturbed heathland habitats, being an unconsolidated mix of decaying plant material punctuated by fine roots and hyphae. Because roots and fungal components were present, enzyme activities in litter reflected the combined influence of both exudates from the microbial community and plant roots. Our 2012 assessment of larval impacts, therefore, focused only on the litter layer because it was expected this would receive waste products immediately subsequent to larval herbivory. The grid quadrat frame was also used to record vegetation data at sampling points, with cover estimated by recording the presence or absence of each species in the 64 grid squares (2 cm x 2 cm).

Insect outbreak assessment, litter sampling and soil respiration

Starting in June 2012, the birch heath area surrounding Abisko Scientific Research Station was subject to a naturally occurring episodic outbreak of autumn moth larvae (*Epirrita autumnata*). The last larval outbreak occurred in 2004 (Morin 2013) and these events have a return interval of ~9 years (Tenow 1972). The larval outbreak of 2012 was therefore anticipated and we exploited this timing to assess how larval impacts further affected phenol oxidase activity and soil surface respiration across this plant community using the same experimental area as 2011. During the peak growing season of 2011 no larvae were found in our experimental vegetation, hence, the impacts of herbivory for 2012 relate to larval foliar

damage in that year only. In our initial evaluations for 2012, the highest larval densities were recorded on 12 July when local mean data showed 266.7 (± 60.6 s.e.) individuals m^{-2} (*n*=10, 0.03 m^2 sample area) in the shrub vegetation. Larval numbers gradually declined after this time point. Occasional periods of rainfall (a mean of 6.4 mm per day during 12-23 July, Abisko Scientific Research Station, *pers. comm.*) will have mobilised the soluble products of larval waste following the outbreak (frass, greenfall and throughfall inputs leached from damaged tissues) into litter and soil. Litter samples for the moth outbreak study were collected on the 23 July 2012, (10 days after peak recorded larval density) when larval numbers had largely subsided (12.1 m^{-2} ± 1.5 s.e.) and when herbivore waste products were expected to have been transferred to the litter, thereby, potentially impacting phenol oxidase activity.

Material in 2012 was collected at 80 points, using a similar intersecting grid co-ordinates approach as 2011 (Online resource 1), with a smaller grid spacing (3.1m) to provide a larger number of sample points. Discrete dominant patches for each of the shrub species were used for the 2012 study, allowing us to specifically target larval damage relationships for individual species, in contrast to the gradual transitions in species abundance across this plant community tested in 2011. In study on phenol oxidase activity in a temperate grassland community, Kardol et al. (2010) used a similar sampling rationale to target individual plant species. Because evergreen and deciduous species have differing shoot morphologies, shoot damage from larval herbivory was defined separately for these distinct plant functional types. The leading shoots at the tip of each branch (henceforth: 'shoots') from each species were examined. The evergreen species (*E. hermaphroditum* and *V. vitis-idaea*) were recorded as 'damaged' if the cuticle of the leading shoot had been consumed, leading to shoot tissue browning. Shoots from the broad-leaved deciduous species (*V. myrtillus* and *V. uliginosum*)

were ‘damaged’ when >25% of the leaf material from any one shoot had been affected. This distinction was used for deciduous species because if their shoots sustained <25% damage, all leaves were typically retained, whereas greater levels of damage always incurred complete loss of all leaves from the affected shoot tip.

We randomly assigned, *a priori*, one of the four shrub species as a ‘target’ for each of the 80 intersecting grid points (n = 20 per species) and at each of these points used the nearest patch dominated by this species. In each case the sample points were <30cm from the grid intersection and where the target species exceeded 50% of the total vegetation cover within a 16x16cm quadrat area (visually assessed using the presence or absence of shoots in 2x2cm grid squares within the quadrat as a reference). The remaining two or three other species were <50% cover in each case and, hence, could be considered ‘sub-dominant’ for that patch. Damage scores for the dominant species in each patch were recorded by counting the proportion of damaged shoots (damaged shoots / total shoots) within the 16 x 16cm quadrat area (25.6 dm^2) and litter was collected by hand from this area. Our area-based shoot assessment method also accounted for the shoot density (shoots dm^{-2}) of the dominant species within the quadrat area (25.6 dm^2) and was a measure of relative species abundance not affected by foliar damage. By contrast, assessment methods for vegetation cover using vegetation area would be affected by foliar damage. Although shoot damage varied greatly at a patch scale (between 1and 100% shoot damage was recorded for all sample points) an assessment of shoot damage across all samples determined that the range of damage for each species was spatially consistent across the experimental vegetation area. The potential for the confounding effect of higher shoot densities to have facilitated greater amounts of shoot damage was also investigated by regression, which was unable to show a relationship.

In situ litter-soil respiration rates were measured across the experimental vegetation area on 5th August 2012 (~21 days after peak insect activity) to determine whether herbivore activity had affected soil surface respiration rates. Respiration rates were measured using a custom made (30mm diameter x 110mm long) PVC soil respiration chamber, fitted to an infra-red gas analyser (IRGA) (EGM-3, PP-systems, Hitchin, UK). This respiration chamber was held to the soil surface at the centre of a vegetation quadrat by opening the shrub canopy and parting all stems to obtain an unvegetated area of litter and soil (henceforth simply, 'soil') below. Pushing firmly on to undisturbed litter material at the soil surface created an air-tight seal . Soil respiration rates, therefore, included a component of plant root respiration. The CO_2-flux (change in CO_2 concentration sec^{-1} cm^{-2}) was calculated using internal IRGA regression algorithms following gas exchange measurement for 120 seconds. A digital thermometer (Cat number 36-1833, Clas Ohlson, Sweden) was used to record soil temperature at the time of measurement. The sampling protocol for soil respiration rate measurement followed a similar spatial (grid) arrangement to that used for litter collection. Vegetation measurements of shoot density and shoot damage for the dominant species were obtained at the time of respiration measurement using shoot counts and a 16 x16cm quadrat frame. Two species were targeted for soil respiration assessment (*E. hermaphroditum* (*n*=22) and *V. myrtillus* (*n*=22)) as, they were the most highly damaged evergreen or deciduous species, despite *V. myrtillus* being of low overall abundance in this plant community. Soil respiration data were collected at 44 intersecting grid points using a wider grid spacing (4.2m) than the 2012 phenol oxidase survey because of the smaller total number of samples taken in this aspect of the study, as only two species were sampled (Online resource 1). However, the number of replicates per species (*n*=22) was higher than the 2012 phenol oxidase assessment (*n*=20 per species) because the soil respiration chamber sampled a

253 smaller area (7.1cm^2) than the 16 x 16 cm quadrat used in the phenol oxidase assessment. .
254 Similar to the above protocol, the most highly abundant (henceforth: 'dominant') target
255 species at each vegetation patch (where >50-90% of the total vegetation cover comprised a
256 dominant target species) was determined by prior random allocation for each intersection
257 point. The dominant patches (16 x 16 cm quadrat area) where always obtained < 30cms from
258 the grid intersection..
259

260 *Litter and soil sample processing*

261 Material collected from the 2011 species composition study comprised the litter layer
262 and O-horizon soil. In 2012, our assessment of larval outbreak impacts used litter only. In the
263 2011 study, soil samples were physically screened by soil layer dissection, so that only the
264 upper 'organic-rich' O-horizon layer was used. The depth of this soil layer varied between
265 locations, so a set soil depth could not be used to partition soil layers. Separation of the O-
266 horizon ensured that only the most active near-surface layer (usually a 0-2cm section depth)
267 of soil was used for analysis, being clearly identifiable from its high organic content. Any
268 visible roots from O-horizon soils or litter samples were removed by hand before further
269 processing. Given the highly organic nature of soil samples taken from a shallow-depth in
270 this habitat, no stones were present. Composite soil samples from a single sample point
271 (~100g) were homogenised in a laboratory bench top blender (Janke and Kunkel IKA
272 Labourtechnik, Germany), as were litter samples (~50g), at full power for 90 seconds until
273 the assay material developed a consistent coarse-ground state (<2 mm particle size). Samples
274 were either transferred immediately to the Abisko Scientific Research Station laboratory for
275 processing, or stored in a controlled temperature room at 5 °C.
276

277 *Laboratory analysis of soil and litter samples*

Soil and litter pH was measured from a 2:1 suspension in distilled water, following the standard methodology of Allen et al. (1974). The percentage field moisture of each sample was obtained by drying ~ 20g of the material at 80˚C for 24 hrs and re-weighing. Analyses of moisture content enabled assays on material at field moisture (phenol-oxidase activity, mobile NH_4^+ and phenolics) to be expressed according to 'equivalent dry weight' and the moisture data were further used as co-variates for some statistical analyses. Phenol oxidase activities were assayed using the method of Pind et al. (1994), with randomly selected duplicate samples processed for procedural precision checking (at 10 % of the total number of samples). These duplicates confirmed a precision of >90%. The previously homogenised litter or soil samples at field moisture were mixed in a 1:9 (soil: water) solution with high-purity filtered water using a vortex mixer for 30 seconds. 3ml of this solution was introduced to 4.5ml of water and 7.5ml of 10 mM *l*-dopamine phenylalanine (L-DOPA). We elected not to use a buffer for this reaction, given that buffers themselves may interfere with enzymatic activity (German et al. 2011) and because we wanted to assess the relative phenol oxidase potential at the native pH for each of our substrates. Furthermore, the pH range of all litter and soil samples fell within 1.2 pH units (4.69-5.93 pH range for all samples in the study), hence, relative pH differences were expected to have only a small influence on phenol oxidase activity. To address the potential influence of pH, however, we used a statistical constraining method (in statistical models with phenol oxidase activity - see further details under 'Statistical analyses'). Phenol oxidase transforms an introduced colourless organic phenol source, L-DOPA, to the red pigment 2-carboxy-2,3,-dihydroindole-5,6-quinone (dicq), with the concentration measured by absorbance from a centrifuged aliquot using spectrophotometry at 460 nm (Thermo Electron Corp., UK), following nine minutes incubation at an ambient laboratory temperature of 20˚C. The molarity of the final solution was calculated according to a constant given by the Beer-Lambert law and the path-length of

the spectrophotometric cuvette (10mm) (Pind et al. 1994). Phenol oxidase activity was, therefore, expressed as μMol dicq (2,3-dihydroindole-5,6-quinone-2-carboxyate) g^{-1} dry weight min^{-1}, typically shortened to μMol dicq g dry wt^{-1} min^{-1}. All sample readings were obtained by first zeroing the spectrophotometer with an unreactive paired soil or litter sample, which was prepared identically, but with distilled water replacing the L-DOPA. All paired zero-corrected sample readings were zeroed from the absorbance of a sample containing only 10 mM L-DOPA and the relevant quantity of water. Organic matter content (2011 samples) was determined in soils and litter by measuring weight loss on sample combustion to ash at 400 ºC for 8 hrs (Allen et al. 1974). This analysis was not repeated on the 2012 litter samples, because the 2011 litter samples consisted largely of organic matter (82-99%) and small differences in this value were not statistically useful for interpreting the results in litter. Water soluble (mobile) NH_4^+ and phenolics were extracted from 2012 litter samples using distilled water following a similar procedure to Allen et al. (1974) for plant-available extraction. 3 ± 0.1 g litter at field moisture was suspended in 25ml water, shaken for one hour and filtered. The extracts were analysed for total NH_4^+ concentration by automated UV-visible colourimetry (Auto Analyzer III, Bran Luebbe, Chicago, IL) and total phenolic concentration (expressed nominally as gallic acid equivalent) was determined by the Folin–Ciocalteu method (Singleton et al. 1999), with solution absorbance measured via UV-visible spectrophotometry (Biomate 5, Thermo-Electron Corporation, Woburn, MA).

Statistical analyses

Data analysis was performed using *R v*2.12.2 (*R* development core team 2011). The data were analysed using generalised linear models (GLM), or generalised linear mixed effects models (GLMM). In general, our analyses tested the relationship of a primary continuous factor (percentage damaged shoots, species cover, or shoot density) on enzyme activities, mobile

328 NH_4^+ and phenolics and/or, soil respiration rate. Physiochemical substrate variables (organic
329 matter content, or moisture content) were also included as interacting co-variates in models to
330 test whether these contributed to relationships with phenol oxidase activity. These substrate
331 co-variates were specifically selected because they have been identified as important controls
332 on phenol-oxidase activity (Sinsabaugh 2008). In the case of phenol oxidase activities,
333 substrate pH was introduced as a 'random effect' using a mixed effects model (GLMM),
334 which statistically constrained the potential influence of pH on the 'fixed effect', phenol
335 oxidase activity. The soil respiration analysis also initially used soil temperature as a model
336 co-variate, but this did not increase the descriptive power of any model according to changes
337 in the Akaike Information Criterion (AIC) (Crawley 2009), so was discarded.
338
339 Multiple co-variates were evaluated in candidate models, using hierarchical stepwise
340 methods which removed co-variates and checked for improvements in AIC values (Crawley
341 2009). All response variables and co-variates were transformed where necessary using
342 logarithmic, square-root, or arc-sin functions (only in the case of percentage data), to gain
343 homogeneity of variance. Improvements in data conformity were confirmed according to
344 changes in the residual deviance values in model outputs.
345

346 # Results

347

348 ## *Plant community composition and phenol oxidase activity*

349 In the 2011 community composition study, *E. hermaphroditum* cover had a strongly positive
350 and significant relationship with phenol oxidase activity in the organic O-horizon soil ($F_{1,28}$ =
351 26.1 P <0.001) (Fig. 2) (Table 1). A significant relationship for this species, with minimal
352 AIC values (suggesting the strongest model), was also detected when organic matter content

353 was included as a co-variate, although the significance of this model was less ($F_{1,28}$ =4.6 *P*
354 <0.05); suggesting that, although the interaction with organic matter was significant, the co-
355 variate only weakly contributed to the statistical model. Litter phenol oxidase activity was not
356 significantly related to any variable in 2011, including *E. hermaphroditum* cover. No other
357 species cover relationships were significantly correlated, either with phenol oxidase activity,
358 organic matter content, substrate pH or substrate moisture content (some non-significant
359 model outputs are not shown in Table 1 for brevity). The soil and litter samples had a
360 consistently acidic pH range, and relatively high organic matter and moisture content (mean
361 values for these variables are given in Table 2).
362

363 *Insect outbreak relationships with phenol oxidase activity, mobile NH_4^+ and*
364 *phenolics, and soil respiration*

365 With the 2012 moth outbreak, damage by larvae occurred to varying degrees in all shrub
366 species. The data were not directly comparable between deciduous and evergreen species, as
367 different definitions of foliar 'damage' were used to account for differences between these
368 morphologically distinct plant functional types. Damage scores from either the two evergreen
369 species or the two deciduous species may be compared, however (Table 3). Of the evergreen
370 species, *E. hermaphroditum* had a greater mean level of damage (55.1± 9.4% mean damage
371 score) than evergreen *V. vitis-idaea* (37.6 ± 7.8% mean damage score), although the
372 difference between these species was non-significant (*P* =0.16). Of the deciduous species, the
373 mean damage levels for *V. myrtillus* (52.4 ± 9.3% mean damage score) and *V. uliginosum*
374 (47.3 ± 9.9% mean damage score) were similar (*P* = 0.71). In *V. myrtillus* patches, a
375 significant negative relationship was detected between phenol oxidase activity and larval
376 damage ($F_{1,18}$ =9.59 *P* <0.01) (Fig. 3). Phenol oxidase activity in the litter beneath *E.*
377 *hermaphroditum*, *V. vitis-idaea* and *V. uliginosum* had no relationship with the level of larval

damage (Table 4, 5), suggesting larval damage had not affected rates of phenol oxidase activity in the litter of these species at the time of sampling. However, in *V. uliginosum* litter larval damage had a strongly negative relationship with mobile NH_4^+ and phenolics (Table 5) and in *V. vitis-idaea* litter, a weak negative relationship with mobile NH_4^+ (Table 4) that was non-significant ($F_{1,18} = 3.59$ $P = 0.07$). Collectively, these results suggest herbivory reduced the availability of NH_4^+ and/or mobile phenolics in the litter of these two *Vaccinium* species at the time of sampling. In a similar direction to the soil phenol oxidase relationship with cover found in 2011, *E. hermaphroditum* shoot density also had a strongly significant positive relationship with soil respiration rates ($F_{1,20} = 12.2$ $P < 0.01$) (Fig. 4, Table 5) not seen in *V. myrtillus*. Soil respiration rates were not significantly affected by the level of larval damage under *E. hermaphroditum* or *V. myrtillus*.

Discussion

We firstly aimed to determine which plant species had the strongest influence on phenol oxidase activity in this plant community, evidencing a positive relationship between *E. hermaphroditum* abundance and phenol oxidase activity in shallow O-horizon soil for 2011. This suggests that, during the peak growing season of a non-outbreak year, soil phenolic decomposition processes in this habitat are a function of plant community composition and such processes are principally influenced by *E. hermaphroditum*, an abundant species in this community. It has been asserted that plant community composition in the Arctic is coupled to below-ground processes (Hobbie 1992; Cornelissen et al. 2001; Dorrepaal et al. 2007) and by finding relationships with the abundance of one species, our findings partly support this. Additive effects from other shrub species in the community may have enhanced the phenol oxidase relationship evident for *E. hermaphroditum* meaning that, more generally,

403 differences in community composition my contribute. Such additive effects may have
404 partially confounded this aspect of the analysis. Specific relationships between plant species
405 composition and phenol oxidase activity have been found in earlier studies on temperate
406 forests (Kourtev et al. 2003; Ushio et al. 2010), although no association has been investigated
407 previously for Arctic ecosystems. In a similar area of sub-Arctic heath to that in our study,
408 Hobbie (1996) showed that levels of polyphenolic lignin compounds in litter directly
409 influenced decomposition activity. Such an association may account for the positive phenol
410 oxidase relationship with *E. hermaphroditum* cover (Table 1). Measurement of soil
411 respiration rates after the larval outbreak of 2012 also showed a positive relationship with *E.*
412 *hermaphroditum* abundance (shoot density) (Table 6), indicating that increased litter-soil
413 biological activity is associated with this species during the peak growing season. In
414 combination, soil respiration and phenol oxidase relationships over two peak growing seasons
415 suggest an elevated level of biotic (root- and microbial) decomposition activity associated
416 with this species. This may be a characteristic feature of *Empetrum* dominated soils, roots and
417 their associated organic material, suggesting adaptations that facilitate localised control of
418 nutrient cycling and enhance the competitiveness of this species. This supports similar
419 conclusions made by Tybirk et al. (2000) in a synthesis of related *Empetrum* research,
420 pointing an allelopathic phenolic compound, batatasin-III present in litter that may also
421 inform high rates of phenol oxidase activity in the present study.
422
423 Our second aim was to determine whether a moth larval outbreak (in 2012) affected
424 litter decomposition processes over an acute timescale during the peak growing season. To
425 achieve this we sampled ~10 and ~21 days after peak larval activity, evidencing some short-
426 term effects of herbivory on decomposition. These are indicators of probable impacts on
427 nutrient dynamics for some species, which may persist in this community, particularly given

the short growing season and the relative sensitivity of this habitat to disturbance. However, we accept that with a limited sampling window, some uncertainty remains as to how transitory the effects we evidence are, or whether longer term impacts would affect other elements of litter-soil carbon or nutrient cycling (e.g. via changes to soil respiration rates due to a reduction in root biomass). In our study phenol oxidase activity was employed as an indicator of changing biological processes in decomposing plant litter (Caldwell, 2005, Kardol et al. 2010) (i.e. a decomposition bioindicator) at the soil surface, following the larval outbreak. Herbivore nutrient inputs were potentially the strongest immediate driver of enzyme activities, owing to hypothesised biochemical disturbance caused by waste inputs to the litter surface (Weedon et al. 2011). In our short-term study, however, only phenol oxidase activity in *V. myrtillus* litter was significantly affected; becoming reduced with increased level of herbivore damage. Soil respiration rates, mobile NH_4^+, or the total mobile phenolic concentration under *V. myrtillus* were unaffected, suggesting that herbivory impacts in the short term were limited to only litter phenol oxidase activity and had not perturbed other elements relating to root activity or microbial nutrient uptake under this species. We hypothesised that enhanced mobility of insect waste inputs would drive such phenol oxidase changes, but this is unlikely because increased availabilities of NH_4^+ or total phenolics were not observed. It is possible that reduced levels of phenol oxidase activity may be accounted for by a decline in root-mycorrhizal exudate productivity following above-ground larval damage. Such above- below-ground linkages from herbivore damage have been previously proposed (Bardgett and Wardle 2003). Lower levels of phenol oxidase activity, therefore, evidence a potential constraint on *V. myrtillus* to mobilise nutrients from litter during the growing season, following foliar damage from larval outbreaks. By contrast, the significant negative relationships in *V. uliginosum* litter between mobile NH_4^+ or total phenolic compounds and larval damage, indicates immobilisation, or enhanced loss by leaching from

453 the litter layer, following herbivory. In this instance a negative relationship for mobile
454 compounds also contradicts our hypothesis. It is possible that some biochemical
455 immobilisation may have occurred due to complexation of NH_4^+ by polyphenolic tannin
456 compounds (Gundale et al. 2010), produced by *V. uliginosum* in response to herbivory.
457 However, such an explanation would not account for the reduction in mobile litter phenolics
458 also observed. NH_4^+ and other available compounds in larval waste may have been
459 biologically immobilised by 'priming' activity in the litter microbial-mycorrhizal community
460 (Lovett et al. 2002). Such an effect would stimulate luxury microbial consumption and,
461 therefore, immobilisation of mobile nutrients (Shen et al. 1984), between the start of the
462 outbreak and the time of sampling. Low-N adapted microbial communities are considered
463 highly competitive for recently mobilised mineral N (Kaye & Hart, 1997) and their foraging
464 ericaceous mycorrhizae can respond rapidly to new additions of mobile organically-bound
465 nutrients (Bajwa et al.1985; Bending & Read 1995). Thus enhanced microbial nutrient
466 immobilisation under *V. uliginosum* is plausible and may explain the reduced mobility of
467 NH_4^+ seen in the litter of this species after larval herbivory. The lack of an equivalent
468 response in *V. myrtillus* may be accounted for by structural differences in the leaves of these
469 two species. *V. uliginosum* typically has greater levels of cell wall-bound phenolics and is
470 closer in leaf structure to evergreen *V. vitis-idaea*, whereas *V. myrtillus* leaves contain greater
471 levels of intra-cellular phenolics (Semerdjieva et al. 2003). Such differences would influence
472 transfer of these waste products to litter, because as higher molecular-weight phenolics are
473 less digestible for moth larvae (Barbehenn and Martin 1992).
474
475 Previously, soil enzyme research has focussed on abiotic drivers, including warming
476 and elevated nitrogen deposition, in disrupting enzyme activity in Arctic soils (Nemergut et
477 al. 2008; Sinsabaugh et al. 2008). Our study indicates that indirect changes via alterations in

478 plant community composition or the frequency of larval herbivory could be important drivers
479 of soil enzyme activity for this region. Because relationships differed between species, our
480 findings support the view that localised control over nutrient cycling is a key trait for Arctic
481 plants such as *Empetrum* (Hobbie 1992; Wookey et al. 2009). Herbivory has been highlighted
482 as an important and previously overlooked disturbance factor for boreal-tundra plant
483 communities at risk from future global change scenarios (Olofsson et al. 2009) and we
484 suggest that biotic interactions such as these, will affect litter and soil decomposition
485 processes differently across related communities according to local variations in community
486 composition. Decomposition processes under *E. hermaphroditum* appear relatively
487 unaffected by moth larval herbivory, however, evidence from our study suggests that
488 decomposition processes and litter nutrient dynamics under the more palatable deciduous
489 species; *V. myrtillus* and *V. uliginosum* are modified by herbivore impacts during the peak
490 growing season. As warmer climatic conditions perturb relationships between plants, soil and
491 primary consumers in the Arctic, the significance of such herbivore interactions with plant
492 community dynamics will intensify. It is important to consider these co-occurring influences
493 when evaluating climate-driven changes to Arctic ecosystems and their stores of organic C.
494
495

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685 understanding their role in the responses of Arctic and alpine ecosystems to environmental
686 change. Glob Change Biol 15: 1153-1172
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688 Figure 1

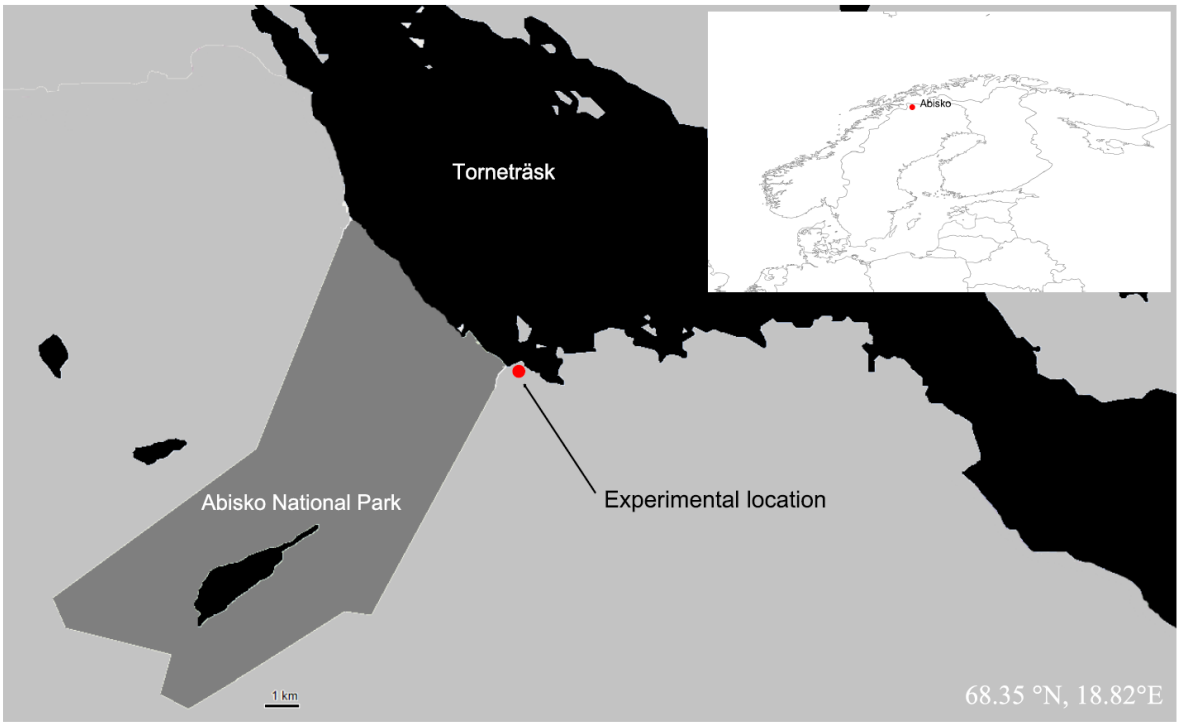

Figure 1: Map depicting location of experimental study area (indicated by circle), beside lake Torneträsk at the Abisko Research Station, northern Sweden (68.35 °N, 18.82°E).

689

690

Figure 2

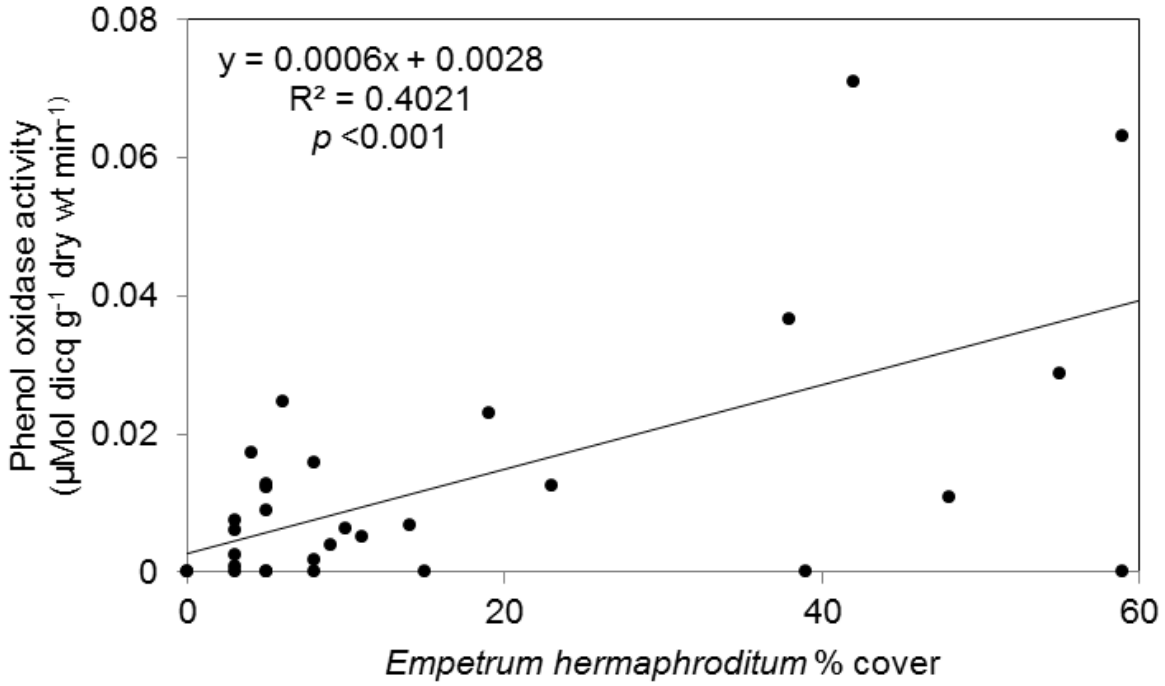

Figure 2: Scatterplot depicting the relationship between *E. hermaphroditum* percentage cover and O-horizon soil phenol oxidase activity. Regression line and R^2 value obtained on untransformed data by linear regression (*n*=28).

Figure 3

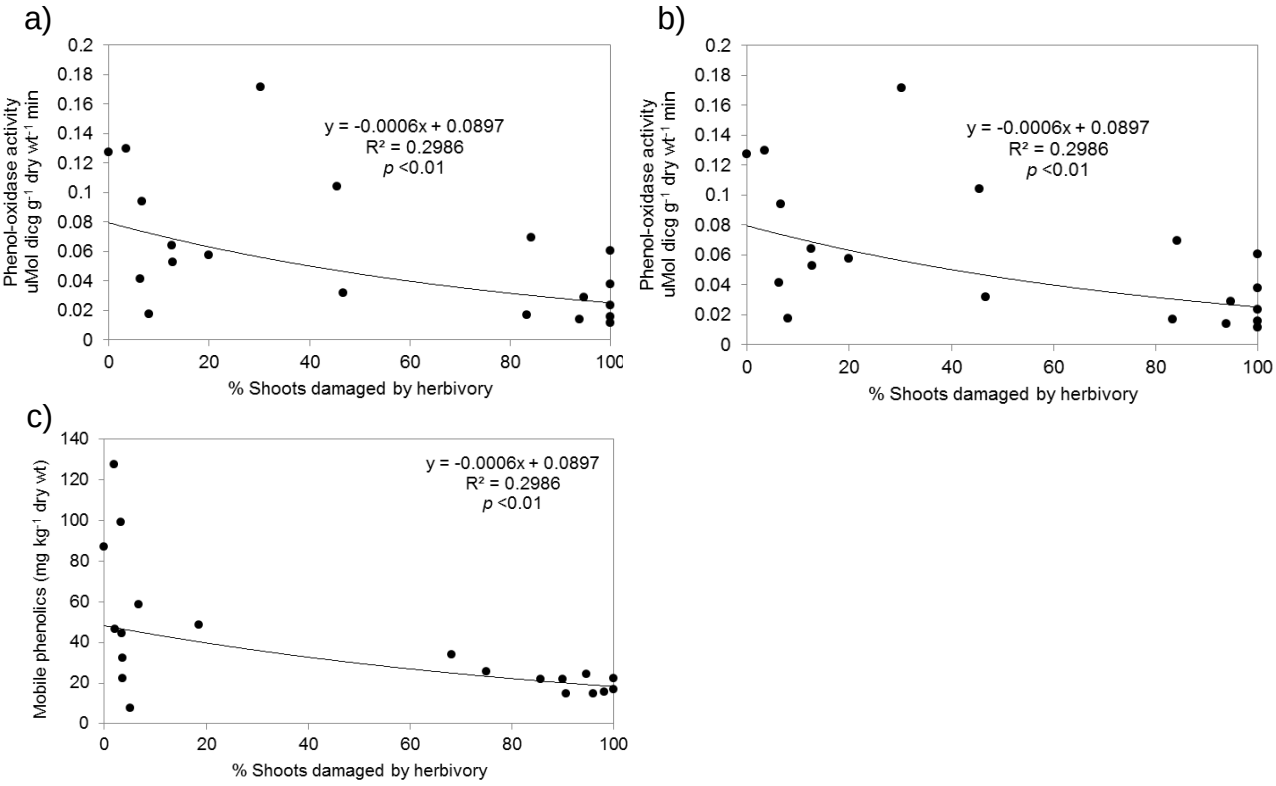

Figure 3: Scatterplot depicting the relationship between percentage shoot damage score and: a) *V. myrtillus* litter phenol oxidase activity, b) *V. uliginosum* litter mobile NH_4^+, c) *V. uliginosum* mobile phenolics. Regression line equation and R^2 value as shown were obtained by regression with an exponential function fitted to values for percentage shoot damage score (*n*=20).

Figure 4

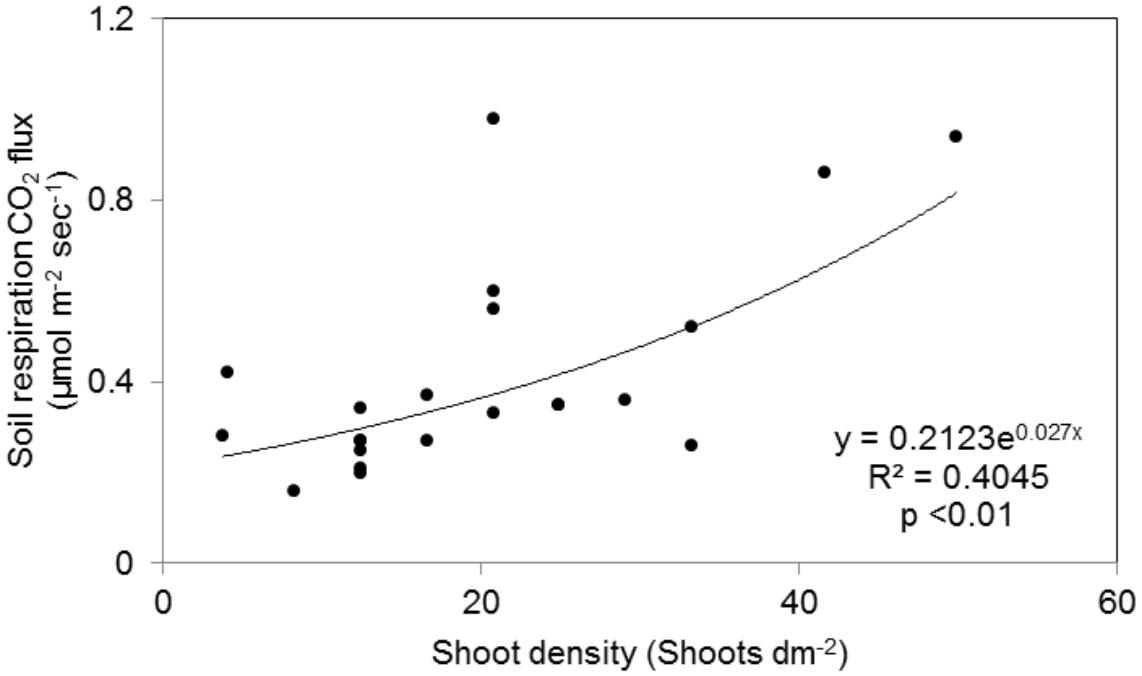

Figure 4: Scatterplot depicting the relationship between *Empetrum hermaphroditum* shoot density score and soil respiration rate. Regression line equation and R^2 value as shown were obtained by by regression with an exponential function fitted to values for shoot density (*n*=22).

Table Legends

Table 1: Statistical summary table for uni- or multivariate GLMM analyses of phenol-oxidase activity in 2011 for (Model 1) litter or (Model 2) O-horizon soil according to *Empetrum hermaphroditum* cover. For brevity, non-significant outputs for all modelled relationships with other species are not shown. Substrate moisture or organic matter were included as interacting variables (as fixed effects). Degrees of freedom for numerator and denominator (df_n, df_d), *F* and *P* values given. *P* values in the table are at * $P < 0.05$, *** $P < 0.001$ levels of statistical significance.

Table 2: Data summary, showing minimum, maximum and mean values for: pH, organic matter content moisture content, mobile NH_4^+ and mobile total phenolics in (1) 2011: mixed-species litter and O-horizon samples and (2) 2012: dominant species patches.

Table 3: Data and statistical summary of 2012 larval foliar damage scores per shrub species with outputs for GLM differences between non-deciduous and deciduous species. Maximum, minimum, and mean (± s.e.) percentage larval damage scores are grouped according to pairs of evergreen and deciduous species, measured 11 days after peak larval activity on the 23rd July 2012.

Table 4: Statistical summary table for uni- and multivariate GLMM analyses of 2012 phenol-oxidase activity, mobile NH_4^+ and mobile total phenolics in litter from patches dominated by evergreen *Empetrum hermaphroditum* and *V. vitis-idaea*, an deciduous *Vaccinium myrtillus* and *V. uliginosum*, showing relationships with herbivory damage (% damaged shoots) and shoot density (shoots dm^{-2}). Substrate and species composition variables were also tested in multi-variate analyses with phenol oxidase activity. Only model outputs with the strongest Akaike information criterion (AIC) value are presented for brevity. Degrees of freedom for numerator and denominator (df_n, df_d), *F* and *P* values in the table are at ** $P < 0.01$ level of statistical significance. AIC values were used for comparing contenting multi-variate models (these models are not shown for brevity). For a near-statistically significant model, the direction of the slope estimate (±) for the modelled relationship between the primary explanatory variable and phenol-oxidase activity is given.

Table 5: Statistical summary table showing univariate GLM analyses of 2012 soil respiration rates in litter from patches dominated by 2 of the dwarf-shrub species in the study, showing relationships with herbivory damage (% damaged shoots) and shoot density (shoots dm^{-2}). Degrees of freedom for numerator and denominator (df_n, df_d), *F* and *P* values given. *P* value in the table is at ** $P < 0.01$ levels of statistical significance. AIC values were used for comparing contenting multivariate models (these weaker models are not shown for brevity). For the statistically significant model, the direction of the slope estimate (±) for the modelled relationship between the primary explanatory variable and soil respiration rate is given.

793

Table 1

Comparison of species cover effects on phenol oxidase activity											
Model 1						Model 2					
Litter phenol oxidase	$d.f._d$	$d.f._n$	*F*	*P*	*AIC*	O-horizon phenol oxidase	$d.f._d$	$d.f._n$	*F*	*P*	*AIC*
Empetrum hermaphroditum											
Cover	28	1	**3.2**	**0.08**	**-103.4**	**Cover**	28	1	**26.1**	*******	**-146.7**
Cover x moisture	24	1	0.1	0.79	-56.3	Cover x moisture	24		1.9	0.18	-111.2
Cover x organic matter	24	1	0.0	0.93	-66.1	**Cover x organic matter**	24	1	**4.6**	*****	**-116.7**
Substrate variables											
Moisture	28	1	2.0	0.17	-93.7	Moisture	28	1	0.0	0.98	-131.7
Organic matter	28	1	1.3	0.26	-97.1	Organic matter	28	1	0.2	0.66	-133.1

794 Table 1: Statistical summary table for uni- or multivariate GLMM analyses of phenol-oxidase
795 activity in 2011 for (Model 1) litter or (Model 2) O-horizon soil according to *Empetrum*
796 *hermaphroditum* cover. For brevity, non-significant outputs for all modelled relationships
797 with other species are not shown. Substrate moisture or organic matter were included as
798 interacting variables (as fixed effects). Degrees of freedom for numerator and denominator
799 (df_n, df_d), *F* and *P* values given. *P* values in the table are at * $P < 0.05$, *** $P < 0.001$ levels
800 of statistical significance.

Table 2

Variable	Minimum	Maximum	Mean (± s.e.)
Mixed species litter (2011)			
pH	4.3	5.7	4.9 ± 0.05
Organic matter content (%)	82.7	99.1	96.3 ± 0.6
Moisture (%)	9.2	86.8	45.6 ± 3.8
Mixed species O-horizon soil (2011)			
pH	4.1	5.9	4.7 ± 0.07
Organic matter content (%)	74.5	99.1	94.8 ± 0.9
Moisture (%)	39.1	77.4	59.6 ± 1.6
Empetrum hermaphroditum litter (2012)			
pH	4.99	5.9	5.5 ± 0.06
Moisture (%)	55.1	71.9	63.8 ± 1.0
Mobile NH_4^+ (mg kg^{-1} dry wt)	5.6	285.8	59.3± 17.1
Mobile phenolics (mg kg^{-1} dry wt)	27.9	189.9	69.6 ± 8.7
Vaccinium vitis-idaea litter (2012)			
pH	4.9	5.8	5.5 ± 0.06
Moisture (%)	32.1	73.8	56.7 ± 2.2
Mobile NH_4^+ (mg kg^{-1} dry wt)	10.2	226.7	60.0 ± 12.7
Mobile phenolics (mg kg^{-1} dry wt)	28.5	117.3	60.0 ± 5.2
V. uliginosum litter (2012)			
pH	4.9	5.9	5.5 ± 0.07
Moisture (%)	52.0	72.4	64.6 ± 1.4
Mobile NH_4^+ (mg kg^{-1} dry wt)	11.6	270.1	66.1 ± 14.9
Mobile phenolics (mg kg^{-1} dry wt)	7.8	127.8	39.3± 7.1
V. myrtillus litter (2012)			
pH	4.9	5.8	5.4 ± 0.06
Moisture (%)	38.7	65.3	53.9 ± 1.7
Mobile NH_4^+ (mg kg^{-1} dry wt)	5.8	239.0	67.7 ± 14.6
Mobile phenolics (mg kg^{-1} dry wt)	22.9	634.5	73.2 ± 30.0

Table 2: Data summary, showing minimum, maximum and mean values for: pH, organic matter content moisture content, mobile NH_4^+ and mobile total phenolics in (1) 2011: mixed-species litter and O-horizon samples and (2) 2012: dominant species patches.

Table 3

Species	Maximum (%)	Minimum (%)	Mean (%± s.e.)	$d.f._d$	*F*	*P*	*AIC*
Empetrum hermaphroditum	100	1.8	55.1± 9.4				
Vaccinium vitis-idaea	89.6	0	37.6 ± 7.8	38	2.04	0.16	41.7
V. myrtillus	100	0	52.4 ± 9.3				
V. uliginosum	100	0	47.3 ± 9.9	38	0.14	0.71	50.0

Table 3: Data and statistical summary of 2012 larval foliar damage scores per shrub species with outputs for GLM differences between non-deciduous and deciduous species. Maximum, minimum, and mean (± s.e.) percentage larval damage scores are grouped according to pairs of evergreen and deciduous species, measured 11 days after peak larval activity on the 23rd July 2012.

Table 4

Relationships in litter for evergreen species		*Slope*	d.f.	*F*	*P*	*AIC*
Empetrum hermaphroditum						
% Damaged shoots:	phenol oxidase activity		16	0.00	0.95	33.8
	Mobile NH_4^+		18	1.39	0.25	29.3
	Mobile total phenolics		18	2.50	0.13	28.2
Shoot density:	phenol oxidase activity		16	2.00	0.18	31.3
	Mobile NH_4^+		18	1.09	0.31	29.6
	Mobile total phenolics		18	0.80	0.38	29.9
Vaccinium vitis-idaea						
% Damaged shoots:	phenol oxidase activity		16	0.31	0.59	27.8
	Mobile NH_4^+	-	18	**3.59**	**0.07**	**22.6**
	Mobile total phenolics		18	0.51	0.49	27.6
Shoot density:	phenol oxidase activity		16	1.16	0.30	25.4
	Mobile NH_4^+		18	0.12	0.73	26.1
	Mobile total phenolics		18	2.47	0.13	20.6
Relationships in litter for deciduous species						
Vaccinium myrtillus						
% Damaged shoots:	**phenol oxidase activity**	-	16	**9.59**	**	**19.1**
	Mobile NH_4^+		18	0.32	0.58	30.5
	Mobile total phenolics		18	0.52	0.41	49.8
Shoot density:	phenol oxidase activity		16	2.09	0.17	26.5
	Mobile NH_4^+		18	0.09	0.77	30.7
	Mobile total phenolics		18	2.51	0.13	48.2
V. uliginosum						
% Damaged shoots:	phenol oxidase activity		16	1.27	0.28	39.6
	Mobile NH_4^+	-	18	**11.1**	**	**14.1**
	Mobile total phenolics	-	18	**10.2**	**	**39.4**
Shoot density:	phenol oxidase activity		16	0.50	0.50	4.85
	Mobile NH_4^+		18	2.49	0.13	21.1
	Mobile total phenolics	+	18	**3.11**	**0.09**	**45.2**

Table 4: Statistical summary table for uni- and multivariate GLMM analyses of 2012 phenol-oxidase activity, mobile NH_4^+ and mobile total phenolics in litter from patches dominated by evergreen *Empetrum hermaphroditum* and *V. vitis-idaea*, an deciduous *Vaccinium myrtillus* and *V. uliginosum*, showing relationships with herbivory damage (% damaged shoots) and shoot density (shoots dm^{-2}). Substrate and species composition variables were also tested in multi-variate analyses with phenol oxidase activity. Only model outputs with the strongest Akaike information criterion (AIC) value are presented for brevity. Degrees of freedom for numerator and denominator (df_n, df_d), *F* and *P* values in the table are at ** $P < 0.01$ level of statistical significance. AIC values were used for comparing contenting multi-variate models (these models are not shown for brevity). For a near-statistically significant model, the direction of the slope estimate (±) for the modelled relationship between the primary explanatory variable and phenol-oxidase activity is given.

Table 5

Relationships with soil respiration	*Slope*	$d.f._d$	*F*	*P*	*AIC*
Empetrum hermaphroditum					
% Damaged shoots		20	1.80	0.19	354
Shoot density	+	20	**12.2**	**	**265**
Vaccinium myrtillus					
% Damaged shoots		20	1.2	0.29	167
Shoot density		20	0.3	0.59	167

Table 5: Statistical summary table showing univariate GLM analyses of 2012 soil respiration rates in litter from patches dominated by two of the dwarf-shrub species in the study, showing relationships with herbivory damage (% damaged shoots) and shoot density (shoots dm^{-2}). Degrees of freedom for numerator and denominator (df_n, df_d), *F* and *P* values given. *P* value in the table is at ** $P < 0.01$ levels of statistical significance. AIC values were used for comparing contenting multivariate models (these weaker models are not shown for brevity). For the statistically significant model, the direction of the slope estimate (±) for the modelled relationship between the primary explanatory variable and soil respiration rate is given.