Running Title: Arctic shrubs and litter decomposition

Shrub encroachment in Arctic tundra: *Betula nana* effects on above- and below-ground litter decomposition

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Abstract

Rapid arctic vegetation change as a result of global warming includes an increase in the cover and biomass of deciduous shrubs. Increases in shrub abundance will result in a proportional increase of shrub litter in the litter community, potentially affecting carbon turnover rates in arctic ecosystems. We investigated the effects of leaf and root litter of a deciduous shrub, *Betula nana*, on decomposition, by examining species-specific decomposition patterns, as well as effects of *Betula* litter on the decomposition of other species. We conducted a two-year decomposition experiment in moist acidic tundra in northern Alaska, where we decomposed three tundra species (*Vaccinium vitis-idaea, Rhododendron palustre,* and *Eriophorum vaginatum*) alone and in combination with *Betula* litter. Decomposition patterns for leaf and root litter were determined using three different measures of decomposition (mass loss, respiration, extracellular enzyme activity). We report faster decomposition of *Betula* leaf litter compared to other species, with support for species differences coming from all three measures of decomposition. Mixing effects were less consistent among the measures, with negative mixing effects shown only for mass loss. In contrast, there were few species differences or mixing effects for root decomposition. Overall, we attribute longer-term litter mass loss patterns in to patterns created by early decomposition processes in the first winter. We note numerous differences for species patterns between leaf and root decomposition, indicating that conclusions from leaf litter experiments should not be extrapolated to below-ground decomposition. The high decomposition rates of *Betula* leaf litter aboveground, and relatively similar decomposition rates of multiple species below, suggest a potential for increases in turnover in the fast-decomposing carbon pool of leaves and fine roots as the dominance of deciduous shrubs in the Arctic increases, but this outcome may be tempered by negative litter mixing effects during the early stages of encroachment.

Key words: moist acidic tundra, litter decomposition, root decomposition, microbial respiration, exoenzyme activity, mixing effects, Arctic shrub encroachment, winter decomposition

Introduction

A consequence of global climate change is a rapidly greening Arctic (e.g. Goetz et al. 2005, Bhatt et al. 2010), largely due to increases in deciduous shrub growth (Tape et al. 2006). Increases in arctic deciduous shrubs resulting from long-term warming (Walker et al. 2006, Sistla et al. 2013) and fertilization experiments (Shaver et al. 2001) in northern Alaska have been accompanied by a decrease in the abundance of evergreen shrubs and graminoids (Gough et al. 2012). Natural increases in shrub abundance have also been accompanied by decreases in species diversity (Wilson and Nilsson 2009, Pajunen et al. 2011), although the largest decreases in cover have been reported for mosses and lichens (Cornelissen et al. 2001). This change in species composition is likely to affect decomposition rates, and thus ecosystem carbon status. The living plant community influences the decomposition environment by changing the decomposition environment (e.g. temperature, soil moisture and nutrients) (McLaren and Turkington 2011) and because species produce litters that vary in chemistry and physical characteristics (Cornelissen 1996) and hence in decomposition rates (Aerts 1997, Preston et al. 2000). For example, the leaves of a deciduous shrub associated with tundra shrub encroachment, *Betula nana* L., may be expected to decompose faster than other tundra species given its relatively high leaf nitrogen (N) content (Chapin and Shaver 1996, Aerts et al. 2006), high specific leaf area (Cornelissen 1996) and the higher rates of N-cycling in *Betula*-dominated tundra soil (Buckeridge et al. 2009) although the high lignin:N ratio in *Betula* may slow decomposition (Hobbie 1996). In experiments, however, the decomposition rates of *Betula* leaves, relative to other species, has varied with both the experiment location and the length of decomposition. *Betula* leaves decomposed slower than leaves of both graminoid (*Eriophorum vaginatum)* and evergreen shrub (*Vaccinium vitis-idaea*) species in lab incubations (Hobbie 1996), slightly faster than these same species when decomposed *in situ* over 2 years*,* and all three species decomposed at similar rates after three (Hobbie and Gough 2004) and four (DeMarco et al. 2014) years of incubation.

Our knowledge of decomposition patterns in the Arctic is based almost entirely on litters from individual species decomposed on their own (e.g. Hobbie 1996, Hobbie and Gough 2004, DeMarco et al. 2014), yet the tundra is a mix of species that may interact in ways not predicted from these single species experiments. For instance, decomposition rates of litter mixtures may be additive (equal to the rate predicted by the decomposition of the individual species) or they may be interactive (the presence of one species alters the decomposition of the others, i.e., mixing effects) (reviewed in Gartner and Cardon 2004, Cardinale et al. 2011). A variety of mechanisms have been proposed for such interactive effects, including changes in the physical environment that affect decomposer abundance and activity (Gartner and Cardon 2004, Hättenschwiler et al. 2005), and nitrogen transfer between different litter types (N-translocation) (Schimel and Hättenschwiler 2007, Handa et al. 2014).

In addition to being based on single species experiments, our understanding of decomposition in the arctic comes mostly from studies of leaf decomposition. In moist acidic tundra in northern Alaska, there is more than twice as much biomass below- than above-ground (Shaver et al. 2014), and although root turnover is slower than that of leaves (Sloan et al. 2013), root contribution to the litter community may be substantial. However, there may be little species-specific variation in root decomposition rates: compared to leaves, roots are less variable morphologically, although there are differences in their chemical composition (such as lignin content or C:N) (Scheffer and Aerts 2000, Birouste et al. 2012). Overall, studies on root decomposition in the tundra are scarce, in particular those that compare decomposition rates of different species (although see Hobbie et al. 2010). This lack of information results in a substantial gap in our ability to predict the effects of increasing deciduous shrub production on decomposition, and thus on C and N cycling, in the Arctic (Myers-Smith et al. 2011).

Finally, much of the litter decomposition in northern ecosystems occurs outside of the short growing season (Hobbie and Chapin 1996, McLaren and Turkington 2010), yet it remains unknown whether mass loss during these colder seasons is due to biological activity (i.e. high microbial activity under snowpack in early winter) or physical processes associated with freeze-thaw (e.g. fragmentation or leaching). Soil microorganisms are active at cold (sub-zero) temperatures (Uchida et al. 2005, McMahon et al. 2009), and C and N fluxes during winter are important to annual budgets in the tundra (Buckeridge and Grogan 2010, Natali et al. 2014). Although current biogeochemical models treat non-growing season processes as essentially slowed-down versions of “normal” growing season, processes may behave differently within, vs. outside, of the growing season because of changes in microbial community structure (e.g. higher fungal biomass in winter (Buckeridge et al. 2013)) and function (e.g. higher biomass specific microbial respiration rates in winter (Lipson et al. 2008)), as well as changes in ecosystem properties (e.g. increases in N-availability and decreases in N-limitation of microbes in winter (McMahon and Schimel 2017)). Accordingly, growing season and non-growing season processes must be treated differently to accurately describe tundra biogeochemistry.

Decomposition can be measured in a variety of ways, and recent approaches have included the pairing of traditional measurements of mass loss with microbial respiration (Uchida et al. 2005, Butenschoen et al. 2011) or microbial exoenzyme activity (Carreiro et al. 2000, Papa et al. 2008, Brandstätter et al. 2013). These methods measure different temporal and physiological components of the decomposition process, which can help elucidate the mechanisms driving decomposition. Mass loss measures the cumulative result of all past decomposition, including both biological (i.e. microbial and mesofaunal consumption and metabolism) and physical (i.e. leaching, freeze-thaw fragmentation) mechanisms and their interaction over time, whereas, microbial respiration and exoenzyme activity are directly biological (variation in which may be driven by physical factors). In addition, microbial respiration on litter is an instantaneous measurement, whereas exoenzyme activity represents both current potential decomposition and can be predictive of future decomposition, because enzymes may be present in the environment long after they are produced (Burns et al. 2013). Since these measures of decomposition differ in their time-scales and the physiological specificity of the processes they capture, combining a variety of methods can inform us about the importance of physical and biological aspects of decomposition throughout the process.

We sought to evaluate how expansion of shrubs (*Betula nana*) may affect decomposition and thus alter C and N cycling in tussock tundra. To assess these changes, we compare species specific decomposition rates of *Betula* with common species which often concomitantly decrease in cover (*Vaccinium vitis-idaea* L.*,* *Rhododendron palustre* (L.) Kron & Judd and *Eriophorum vaginatum* L.), using both leaf and root litter in an *in situ* experiment. We focused on the decomposition of leaves and fine roots, which turn over relatively quickly and are a significant component of the C and N inputs in this system. Substantial increases in woody stem and rhizome litter are likely to occur with shrub encroachment, but as they turn over much more slowly, we did not examine them in this experiment. We examine the effects of *Betula* litter on the decomposition of other species in litter mixtures and use three different measures of litter decomposition (mass loss, respiration and exoenzyme activity). We hypothesized that:

1. The relatively high quality *Betula* leaf litter will both decompose faster than and accelerate the decomposition of other species in mixtures (i.e., positive mixing effects);
2. For roots, species differences in decomposition rates will be smaller than for leaves, and as a result we do not expect mixing effects for root litter;
3. Mass loss and physical decomposition will be largest during the first winter, but differences between species will be larger during the following summer, as warmer temperatures enhance microbial litter decomposition.
4. Differences among the three measures of decomposition (mass loss, respiration and exoenzyme activity) in how they represent patterns of decomposition between species will increase with time.

Materials and Methods

Site Description

The experiment was conducted at the Arctic LTER site at Toolik Field Station in the northern foothills of the Brooks Range in Alaska (68° 38'N, 149° 43'W, elevation 760 m). The vegetation community is moist acidic tussock tundra, dominated by the sedge *Eriophorum vaginatum* with deciduous (*Betula nana)* and evergreen shrubs (*Rhododendron palustre*, *Vaccinium vitis-idaea*) roughly equal in abundance, and mosses forming nearly continuous cover (Shaver and Chapin 1991). The soil is a Typic Aquaturbel, with an active layer ca. 50 cm thick. The growing season lasts 10-12 weeks, beginning in early June. Our experiment was conducted within permanent plots that receive no experimental treatment, approximately 800 m SW of Toolik Field Station. We used a single 5 x 20 m plot from each of three replicate blocks, separated by minimum 20 m.

Decomposition Experiment

Senesced leaf material from *Betula*, *Eriophorum*, *Rhododendron,* and *Vaccinium* was collected in late-August 2010 from a ca. 50 x 50 m area of moist acidic tundra, adjacent to the plots described above, ensuring that leaves were collected from multiple individuals. Senesced but attached *Betula, Rhododendron, and Vaccinium* leaves were collected by hand from live plants. For the evergreen species *Rhododendron* and *Vaccinium,* we collected only leaves that had changed color and were attached to live stems. For *Eriophorum,* the current year's senescing tillers were selected and green material was removed from leaves before processing.

Root material was collected in late-July 2010 from an area of moist acidic tundra measuring ca. 100 x 100 m, approximately 1 km from the experimental plots, again ensuring that root material was collected from multiple individuals. Root collection was species specific and only roots attached to a living plant were collected. Because freshly senesced root material is difficult to distinguish from older material (Ostertag and Hobbie 1999), live, rather than senesced, roots were used to create litter bags. Roots were washed free of soil, and the fine root (0.5 – 2 mm diameter) size class selected. For *Eriophorum,* all collected material was roots, but for other species we did not differentiate between rhizomes and roots and refer to this material as roots for simplicity. Subsamples of initial root and leaf material were dried, ground, and processed for total C and N content using a dry combustion C and N analyzer (Thermo Scientific 2000 Elemental Analyzer) and for lignin content (ANKOM fiber analyzer).

Litter was dried at 40°C for 48 h, well mixed, and then sub-sampled for litter bag creation. Litter was placed in 8 x 8 cm litter bags made from 1mm nylon mesh. Leaf and root bags were created separately. For both leaves and roots, each species was decomposed both singly and in combination with *Betula,* resulting in 7 species combinations. Species mixtures are abbreviated as BE, BR and BV, where "B" refers to *Betula*, "E" to *Eriophorum,* "R" to *Rhododendron*" and "V" to *Vaccinium*. Leaf bags contained either 1g of a single species, or 0.5g each of a single species mixed with 0.5 g *Betula* litter. *Eriophorum* leaves were cut into 7 cm lengths to fit in the litter bags, while entire leaves were used for other species. Root bags contained 0.5 g litter of a single species, or 0.25g each of a single species and roots of *Betula;* all roots were cut into 7 cm lengths.

Litter bags were installed 20-22 September 2010. For both leaf and root bags, one bag from each of the 7 species combination were attached together on a string and 12 replicate strings were placed in each plot. Leaf litter bags were placed in plots just below (ca. 2-5 cm) the moss surface, as the small leaves of these plants often migrate down into the moss during the decomposition process. Root litter bags were buried 8-10 cm below the surface. We deployed Thermochron iButtons (model DS1921G, Maxim, San Jose, CA) in each plot from September 2010 to August 2012 at 5cm and 10cm below the surface to measure soil temperature associated with leaf and root litter decomposition, respectively. Temperatures were logged every 255 minutes, in 0.5 ^{o}C increments at a 2.0 ^{o}C resolution. Four replicates (randomly chosen) were sampled from each of the three plots at each of the three sampling dates: May 2011 (over-winter – bags were collected immediately after snow melt or when the soil had thawed to 10 cm depth for leaf and root bags respectively), August 2011 (1 year) and August 2012 (2 years).

After collection, the content of each bag was cleaned of foreign material (moss, ingrown roots etc.). For each sampling date, two replicates per species combination for both leaves and roots were immediately frozen at -20 °C and transported to University of California at Santa Barbara for enzyme analysis (described below). The two additional replicates were used for respiration, mass loss and C and N measurements.

Respiration Measurements

Respiration samples were individually placed in 90 ml polypropylene containers and incubated for 6 days in a biological incubator (Geneva Scientific I-36VL, Geneva Scientific LLC, WI, USA), with incubation beginning at 5 °C and increasing 5 °C every 2 days. Litter respiration data from the 15 °C incubation is used in this analysis, while temperature sensitivity of litter respiration will be presented elsewhere. Litter respiration was measured in a lab at Toolik Field Station with an open portable gas exchange system (Li-Cor 6400, Li-Cor Inc, Lincoln, USA), fitted with a custom 350 ml chamber. Each sample measurement lasted 7-10 minutes, and sample temperature was maintained at ca. 15 °C throughout using coolers and icepacks as required.

Because litter decomposition may be sensitive to moisture (e.g. Schimel et al. 1999, Makkonen et al. 2012), water was added to samples to standardize their moisture content. We performed a separate study on the influence of litter moisture on leaf litter respiration which showed little influence of moisture on respiration above 2 g H_2O g^{-1} dry litter (Appendix S1). Since the average incubated sample moisture ranged from 3.2 - 4.2 g H_2O g^{-1}, respiration should not have been affected by small water content differences among the samples.

Mass Loss and Litter C and N

Following respiration trials, litter was dried at 50 °C for 48 hours and weighed to determine proportional mass loss from initial litter (cumulative mass loss). Material was ground and processed for % C and N as above. We then calculated C and N content of leaf/root tissue as g-1 C or N sample, calculated as %C or N x g-1 leaf/root tissue remaining at each sampling. Leaf litter was analyzed separately for each species, including separate measures for component species of mixtures. Because roots could not be accurately identified to species post-decomposition, root tissue was analyzed per species treatment, analyzing the tissue from either single species treatments or root mixtures as a whole.

Microbial Exoenzyme Activity

Microbial extracellular enzyme (exoenzyme) activities were measured on the two remaining replicates from each plot on samples from the first two samplings only (over-winter and 1 year decomposition). Material from replicate samples within the same plot were pooled before analysis (thus different litter compositions were replicated at the plot level only; n=3 for each sampling). Frozen samples were thawed immediately prior to enzyme assays. We assayed the activity of a suite of hydrolytic enzymes that acquire carbon, nitrogen and phosphorous at the terminal stages of organic matter decomposition: cellulose-degrading β-glucosidase and cellobiohydrolase, hemicellulose-degrading β -xylosidase, carbohydrate-degrading α-glucosidase, chitin-degrading N-acetyl-glucosaminidase (NAG) and phosphatase.

Exoenzyme methodology followed that of Sinsabaugh et al. (2003). Leaf litter (2-4 g fresh mass) or root litter (1-3 g) was blended with pH 5 acetate buffer and pipetted into 96-well plates, with eight replicates per soil. Fluorescing, 4-methylum-belliferone (MUB) tagged substrate (β -D-glucoside, β -D-cellobioside, β -D-xyloside, β -D-glucoside, N-acetyl-α-D-glucosaminide and phosphate) was added. The assays were incubated at 5 ^{o}C in the dark within the linear range of the reaction (2-13 h), then the reaction was stopped by adding NaOH. Sample fluorescence (i.e. cleaved substrate) was read with a TECAN Infinite Pro 200 plate reader (Tecan

Group Ltd., Männedorf, Switzerland) at 365 nm excitation, 450 nm emission. For each substrate, we measured the background fluorescence of soils and substrate and the quenching of MUB by soils, and used standard curves of MUB to calculate of the rate of substrate hydrolyzed. The NAG assay was only successful for roots, therefore leaf results for NAG are not presented.

Statistical Analyses

Leaf and root litter were analyzed separately for all variables. Mass loss and respiration were both averaged across the two within-plot replicates before analysis. Enzyme activities were pooled across the 6 enzymes to provide an overall hydrolytic enzyme response because they generally followed the same pattern by species monoculture (averaged to not overinflate degrees of freedom). For species effects, enzymes were standardized (activity/maximum activity) before pooling. Statistical analyses of standardized, pooled enzyme rates are presented, whereas figures illustrate standardized, un-pooled enzyme rates to reveal response variation by enzyme. Unstandardized, un-pooled enzyme values (ranges) and statistics are presented in Appendix S2.

Species Effects: Species differences in mass loss, respiration and enzyme activity were each analyzed using an ANOVA using single species as treatment levels, with separate analyses for each sampling date. Significant species effects were further explored using Tukey's comparison of means. In addition to cumulative mass loss, we also examined the effects of season (winter vs. growing season) on mass loss during the first year, where mass loss during the first winter is calculated as:

$$ML\ 1st\ winter\ =\ Mass_i - Mass_{thaw}$$

and mass loss during the growing season is calculated as:

$$ML\ growing\ season\ =\ Mass_{thaw} - Mass_{fall}$$

Where ML is mass loss, Mass is the mass of the litter (g), i is pre-incubation (Sept 2010), thaw is May 2011, and fall is Sept 2011.

Mixing Effects: To examine for the presence of mixing effects in litter mixtures, we calculated the deviation from expected mass loss/respiration/enzyme activity based on single species rates:

$$Mixing\ Effects = \frac{Observed - Expected}{Expected}$$

Deviation from expected is referred to as 'mixing effects' hereafter. For mass loss, the expected values are the averages of the mass for species decomposed alone for both species in the mixture. Expected respiration rates and enzyme activity values for leaf litter were calculated similarly, averaging single species rates, which were standardized by their observed mass in mixture (thus isolating the mixing effects of respiration or enzyme activity from mixing effects due to changes in mass). For root litter, the latter was not possible since species-specific mass could not be obtained for the mixtures. Instead, these expected values were the averaged single species rates of both species, assuming that for these root mixtures there were no mixing effects on mass loss for roots, an assumption which is supported by our results. We compared the mean mixing effect against a mean of zero using a one-sample t-test for each species combination. A value significantly different from zero indicates interactive effects of species mixing on decomposition (mixtures promote or inhibit decomposition over the sum of the two single species alone).

For leaf mass loss, as we could determine the post-decomposition mass of the individual species within each mixture, we analyzed species-specific decomposition within species combinations using a nested ANOVA, with species nested within litter mixture (McLaren and Turkington 2010). Tukey's comparison of means was used to examine species decomposition rates within and between species mixtures.

The % gain or loss in C or N from litter content is expressed as the % difference from the initial g C or N content for each litter type (calculated as %C or N x g^{-1} leaf/root tissue) at each sampling date. For leaf litter, each species was analyzed independently. For each species, the effect of treatment (monoculture vs. mixture) on relative changes in C or N were analyzed using a one-way ANOVA. When litter treatment was significant (indicating a mixing effect), the relative change in C or N was compared with zero using a one-sample t-test independently for each litter treatment. For each species, when there was no significant effect of mixing on litter gain or loss, t-tests were conducted across litter treatments. The relative changes in C or N for root species combination were analyzed using an ANOVA, followed by a t-test for each species combination.

Statistical analysis were conducted using JMP statistical software (2012 SAS Institute, Cary, NC, USA).

Results

Before decomposition, initial C:N ratios for leaves were highest in *Eriophorum* and lowest in *Rhododendron*, because initial %N was lowest in *Eriophorum* and highest in *Rhododendron*. For roots, initial C:N ratios were highest in *Rhododendron; Eriophorum* and *Rhododendron* roots had similarly low N, but C in *Eriophorum* was also low. Lignin content was highest in *Betula* for both roots and leaves (Table 1).

Leaf Decomposition- Species Effects

For cumulative mass loss, there were species effects in each sampling period (Table 2, Fig. 1a-c). *Betula* litter generally had the highest rates of mass loss, followed by *Rhododendron* and *Vaccinium*, and finally by *Eriophorum* which decomposed the slowest. When seasonal mass loss was examined, during the winter, *Betula* lost at least twice as much mass as any other species ($F_{6,41}$=44.03, p<0.001; Fig. 2a slopes differ). In contrast, leaf litter from all species decomposed at the same rate during the growing season ($F_{6,41}$=1.45, p=0.22; Fig. 2a slopes parallel).

Post-winter respiration rates were highest for *Betula*, ca. twice as high as rates for *Vaccinium* or *Eriophorum* (Table 2, Fig. 1d). However, differences between species in respiration decreased with time and there were no differences among species after two years (Table 2, Fig. 1e-f). Overall, there was no significant difference between species for pooled exoenzyme activity on leaves, although activity tended to be higher on *Betula* litter (Table 2, Fig. 1g-h) and for the four C-hydrolyzing enzymes, was higher on *Betula* after winter and after the first growing season (Appendix S2).

Leaf Decomposition – Mixing Effects

For mass loss, there were negative effects of mixing at all three samplings (Fig. 3a-c, Appendix S3). Both BV and BE exhibited negative mixing effects post-winter and after 1 year, and BR after 2 years. For BV and BE, the magnitude of mixing effects decreased with time, from ca. 30% slower post-winter to 20% slower after 1 year and after 2 years there was no longer a detectable mixing effect for these species combinations (Fig. 3a-c) These negative mixing effects on mass loss were caused by slower decomposition of *Betula* in mixture than when decomposed on its own, particularly at earlier stages of decomposition (Fig. 4; Litter composition (species): winter: $F_{3,50}$=19.83, p<0.001; 1 year: $F_{3,50}$=11.36, p<0.001; 2 year: $F_{3,50}$=8.80, p<0.001). The other species did not differ between monoculture and mixture at any of the sampling times (Fig. 4). Contrary to mass loss, there were very few significant mixing effects for respiration (Fig., 3d-f, Appendix S3). During the post-winter sampling, there was a positive mixing effect for BV, but not for the other two mixtures. There were also few mixing effects for enzyme activity (Fig. 3g-h, Appendix S3); there was a negative averaged hydrolytic mixing effect for BR, but only during the post-winter sampling. For individual enzymes, α-glucosidase activity decreased at the end of the growing season on BV and BE (Appendix S2).

Leaf Decomposition – Loss or Gain in C and N

Decomposing in mixture versus alone affected the %N gained or lost from initial litter tissue only for *Rhododendron* and only after the 1 year sampling (Fig 5a-c; Table 3). *Rhododendron* did not lose or gain N when decomposed alone or in mixture during the winter, but after 1 year of decomposition lost ca. 3 times more N when decomposed with *Betula* than when decomposed alone, and after 2 years lost N both in mixture and alone (Appendix S4). For the other three species the %N gained or lost form initial litter tissue did not depend on decomposing alone or in mixture and thus N loss/gain was averaged across treatments (monocultures and mixtures) for analyses (Fig. 5a-c; Table 3). *Betula* lost N but only after 2 years of decomposition (Appendix S4). *Vaccinium* did not lose or gain N after decomposing for the winter or for 1 year but lost N after 2 years of decomposition (Appendix S4). Finally, *Eriophorum* gained N after decomposing for the winter, 1 year, and 2 years (Appendix S4). Decomposing in mixture versus alone affected the %C gained or lost from initial litter tissue for *Betula, Rhododendron* and *Vaccinium* only in the post-winter sampling (Appendix S4) because leaves lost more C when decomposed in mixture than alone (Appendix S4). At the 1 and 2 year samplings, all species significantly lost C, but the amount lost did not depend on whether they were decomposed alone or in mixture (Appendix S4).

Root Decomposition – Species Effects

There were fewer effects of species on cumulative root litter mass loss as compared to leaves (Table 2, Fig. 6a-c). After 1year of decomposition, *Rhododendron* lost less mass than either

Betula or *Eriophorum,* although mass loss did not differ between species for the other two sampling periods. When mass loss was examined seasonally, similar to seasonal patterns for leaf decomposition, there was a marginally significant interaction between litter species and season ($F_{3,23}$=2.86, p=0.07), because *Rhododendron* roots decomposed more slowly than other species in the winter ($F_{3,11}$=3.88, p=0.06) but not in the summer ($F_{6,70}$=1.11, p=0.40) (Fig. 2b). Similar to effects on mass loss, there are no effects of root species composition on respiration (Table 2, Fig. 6d-f). Exoenzyme activity, in contrast, differed between litter compositions post-winter and after 1 year, because *Eriophorum* had higher activity than other species both for pooled exoenzyme activity (Table 2, Fig. 6g-h) and enzymes individually (Appendix S2)

Root Decomposition – Mixing Effects

There were no mixing effects on mass loss, respiration or averaged enzyme activity for any species composition (Fig. 7 a-f, Appendix S3). For individual enzymes, only β-glucosidase activity decreased for the B-R mixture (Appendix S2.)

Root Decomposition – Loss or Gain in C and N

The proportion of root N lost or gained during decomposition varied by litter composition (Fi.g 5, Table 3). *Rhododendron* and *Vaccinium* significantly lost N post winter and after 1 year, *Betula*, *Rhododendron* and *Vaccinium* all lost N (Appendix S4). However, after 2 years only *Vaccinium* lost N (Appendix S4). The proportion of root C lost or gained did not vary with litter composition (Appendix S4). Only BE had significantly lost C at the post-winter sampling, whereas all species combinations had lost C by the 1 and 2 year sampling (Appendix S4).

Discussion

Our goal was to understand potential effects of *Betula* encroachment on litter decomposition in arctic tundra, particularly the role of litter mixing and seasonality. *Betula* leaves decomposed faster than other species when decomposed on their own, but when mixed with other species the decomposition of *Betula* slowed (i.e., negative mixing effects), implying that single-species decomposition is a poor predictor for the often mixed-litter decomposition that occurs *in situ*. Root decomposition, in contrast, displayed few species differences and no mixing effects. Our three methods of assaying decomposition agreed with each other for relative differences between species and pointed to strong species effects on decomposition during winter. Interestingly, the three methods differed from each other in their assessment of mixing effects, implying that controls on mixed-litter decomposition are not predictable by microbially-specific methods.

Betula effects on leaf decomposition (Hypothesis 1)

Mass loss for *Betula* leaf litter was consistently higher than for other species, which supports the first component of our first hypothesis, and implies faster turnover of leaf litter C and N in communities with higher proportional deciduous shrub cover. All three measures of decomposition showed faster decomposition of *Betula*, at least for earlier samplings, which parallels some studies (Hobbie and Gough 2004) but contrasts with others (Hobbie 1996, Cornelissen 2007). Direct comparisons with these studies are difficult, however, due to different experimental designs (lab study (Hobbie 1996) vs. field incubations (Cornelissen 2007, our study)), length of decomposition period (21 weeks (Hobbie 1996) vs 2 years (Cornelissen 2007, our study)) and species used (*Betula nana* (Hobbie 1996, our study) vs 11 deciduous shrub species (Cornelissen 2007)). Further, although species differences are strong at the end of our experiment (2 years), these species patterns may not persist over the long-term. Although Hobbie and Gough (2004) show faster decomposition by *Betula* in the first 2 years of their experiment, the decomposition rate for *Betula* slowed in year 3 while the other species continued decomposing at the same rate, resulting in no difference between species after 3 years. DeMarco et al. (2014) also describe no species differences in decomposition rate after 5 years. Finally, our experiment investigated the rapidly decomposing leaf litter and fine roots but did not assay slow decomposing woody tissue that will also increase in abundance with shrub encroachment.

We suggest that species differences in these early stages of decomposition are driven at least in part by variation in traits such as specific leaf area (SLA): *Betula* has nearly three times higher SLA compared with *Vaccinnium* and *Rhododendron*, and twice as high as *Eriophorum* (Shaver et al. 2001). Although litter mass loss is often reported to inversely correlate with litter initial C:N (e.g. Zhang et al. 2008), lignin content (Aerts 1997), and lignin:N (Melillo et al. 1982), our species' decomposition rates did not follow these patterns. Differences in reported lignin and lignin:N between ours and other studies could help explain these contrasting results. For example, both Hobbie (1996) and DeMarco et al (2014) report higher lignin:N for *Betula* than *Eriophorum¸* whereas in our study the ratios are relatively similar. Other possible drivers of these species patterns are concentration of phenolics which are suggested to inhibit decomposition (Freeman et al. 2004). However, both the deciduous and evergreen species used here have similar phenolic concentrations (Hobbie 1996) yet differ in their decomposition rates.

Combining *Betula* litter with other species consistently resulted in negative mixing effects for mass loss, contradicting the second component of our first hypothesis. Not only did mixtures decompose more slowly than expected, these negative mixing effects were caused by slower decomposition of *Betula* in mixture, rather than an effect of *Betula* on associated species. Therefore, although our species-specific results indicate that increases in *Betula* litter may result in faster litter turnover, this is tempered by the influence of negative mixing effects. N-translocation (transfer of nitrogen between decomposing species) did not appear to explain mixing effects on mass loss. Although by the final sampling the post-decomposition N content of litter in the *Betula-Eriophorum* mixture does suggest N translocation (simultaneous N increases in *Eriophorum* and decreases in *Betula,* with a trend towards larger increases in N in *Eriophorum* when in mixture*;* analysis described by Handa et al. 2014), by this stage of decomposition mixing effects had disappeared. Because our microbial specific methods, respiration and exoenzyme activity, did not show negative mixing effects, physical differences between litter types (such as differences in SLA) may be responsible for the mass loss mixing effects. As such, microbial C-mineralization and exoenzyme activity may not be good predictors of mass loss mixing effects.

Betula effects on root decomposition (Hypothesis 2)

Consistent with our second hypothesis, both root litter mass loss and respiration revealed few species differences, with only *Rhododendron* roots decomposing measurably slower and only at a single time point. Root decay rates are thought to be mostly determined by chemical quality instead of environmental conditions, because their decomposition environment in the soil is relatively buffered from environmental extremes (Silver and Miya 2001). However, our measures of chemical quality, initial C:N (highest in *Eriophorum*) and lignin:N (highest in *Betula)*, cannot explain the slow decomposition of *Rhododendron*. Instead, slower decomposition of *Rhododendron* roots may have been driven by differences in root thickness and the resulting decrease in surface area: volume. We used fine roots (< 2mm) for all species but *Rhododendron* roots were thicker than other species (pers. obs.).

Also in accordance with our second hypothesis, there were no root litter mixing effects for any of the three measures of decomposition. Although there have been no studies on mixing effects for root decomposition in the field, in a lab incubation, de Graaff et al. (2011) reported higher respiration rates for decomposing roots when mixed together than alone. Further, Robinson et al. (1999) reported both positive and negative mixing effects but concluded that because mixing effects were small (<10%) they were not likely biologically significant. Overall, these studies, and ours, indicate a low potential for mixing effects on root decomposition.

Contrasts between leaf and root decomposition

We found less variation in root than leaf decomposition among species, seasons, and measures of decomposition. We found strong and persistent species effects on mass loss in leaf litter, with *Betula* decomposing faster than other species, while there were few species effects in roots. A recent meta-analysis (Freschet et al. 2013) concluded that decomposition rates of leaves and fine roots globally are coordinated across species, suggesting that the traits responsible for litter decomposability are correlated across tissue types. Perhaps this global correlation holds true for large scale studies, but is not necessarily observed in more narrowly focused single location studies with a select number of species (e.g. this study and Hobbie et al. 2010).

Seasonal differences in decomposition (Hypothesis 3)

Because cold temperatures limit microbial activity outside the growing season, we expected species-specific effects primarily on mass loss in the first winter (i.e. losses due to both microbial and physical drivers), and then species-specific effects on enzymes and respiration (i.e. from microbial drivers alone) to become more active with increased temperatures during the first growing season (Hypothesis 3). In general, early stage decomposition is fastest, because it is dominated by soluble C loss (Aber et al. 1990), which may be physically (i.e. leaching, freeze-thaw fragmentation) and microbially (i.e. polymer breakdown via enzymes) driven. Species-specific differences in mass loss, respiration and enzyme activity (i.e. both physical and microbial drivers) were all determined over the first winter, and did not differ further in later seasons, which is contrary to Hypothesis 3. Further, species specific differences for post-winter litter respiration (microbial), extracellular enzyme activity (microbial) and winter mass loss (microbial and physical) were strikingly similar, suggesting that the decomposition mass loss patterns in this first winter are most likely driven by microbial (*e.g.* Uchida et al. 2005) rather than only physical controls (*e.g.* Bokhorst et al. 2009).

We speculate that differences between species in their decomposition during the first winter were likely driven, at least in part, by differences in the decomposition of the more soluble components of their litter. A proportionally higher microbial contribution early in decomposition could represent rapid microbial processing of the soluble fraction of the litter (Cotrufo 2015). Although *Betula, Rhododendron* and *Vaccinium* have all been reported to have similarly high water-soluble sugar content (Hobbie 1996), the higher SLA of *Betula leaves* may have resulted in higher accessibility of these soluble components by the microbial community, driving the faster decomposition patterns. We also suggest that many of these decomposition differences develop during the 'shoulder seasons', i.e., the periods right at the beginning and the end of the snow-covered period; Although soil microorganisms are active at cold (sub-zero) temperatures (McMahon et al. 2009), the deep cold period of tundra winter precludes substantial microbial activity, yet soil temperatures in early and late winter are warm enough to support substantial organic matter turnover and microbial growth (McMahon et al. 2009, Buckeridge et al. 2013). As leaf decomposition progresses, and these soluble components are lost from the litter tissue, differences between the species in decomposition may become minimized, resulting in the similar decomposition of different species during the growing season that we describe. We did not measure changes in the soluble components of these species over time, and note that less than 20% of litter mass had been lost by the beginning of the growing season (and less than10% for most species), suggesting that we were still in relatively early stages of decomposition, not only at the end of the first winter, but also at the end of the experiment. We encourage longer-term seasonally-delimited decomposition experiments where leaf chemistry is examined over time to tease apart seasonal differences from those driven by early vs. late stage decomposition.

Similarities and differences among the three measures of decomposition (Hypothesis 4)

We found broad similarities in patterns of species-specific decomposition among the three measures of decomposition; all three initially showed the highest decomposition rates in *Betula* leaf litter and the lowest in *Eriophorum*. Similarly, for root decomposition all methods reflected marginally lower rates of decomposition in *Rhododendron* and few other differences between species. These similarities, however, diminished with time, in particular for leaf decomposition where species differences decreased over time for respiration but persisted with the other two methods. This supports our fourth hypothesis, and highlights both the different time frames (cumulative vs. instantaneous) and the microbial physiological specificity that the different measures represent. The resemblance of initial leaf respiration patterns to longer-term mass loss parallels findings of Aerts (1997), where initial litter respiration differences among species predicted long-term decomposition differences. It also emphasizes that only *early* respiration, and not just respiration at any point in a decomposition experiment may be a proxy for longer-term litter mass loss. Further, for root decomposition, heightened enzyme activity in *Eriophorum* was not predictive of larger mass loss at successive time points. We conclude that these more microbially-constrained measures cannot be used to represent litter mass loss as a whole.

Conclusions

Our study provides two strong and contrasting conclusions regarding shrub encroachment. The high decomposition rates of *Betula* leaf litter aboveground, and relatively similar decomposition rates of different species' roots below-ground, suggest potential increases in C turnover as the dominance of this deciduous shrub in the Arctic increases. However, tundra litter species decompose in mixture, and the negative mixing effects that we observed among species in leaf decomposition are likely to temper the strong *Betula* effect, at least in the early stages of shrub encroachment. Until *Betula* becomes dominant enough to "escape" the negative mixing effects with other litter species, decomposition rates are likely to remain constrained. We note, however, that our results focus only on the fast-decomposing C pool of leaves and fine roots, whereas longer-term decomposition patterns are likely to be dominated by the increase in slow-decomposing woody tissue accompanying increases in shrub abundance.

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Table 1 Initial C:N ratio, %N, Acid detergent lignin (ADL) and ADL:%N ratio in leaf and root litter tissue for the four litter species used in this study (mean ± SE, n=5)

	Leaves				Roots			
Species	C:N	%N	% ADL	ADL:N	C:N	%N	% ADL	ADL:N
Betula	56.9 ± 0.7	0.9 ± 0.01	19.3 ± 0.3	21.4 ± 0.2	66.2 ± 2.8	0.8 ± 0.03	40.1 ± 2.0	53.4 ± 1.4
Eriophorum	98.2 ± 2.3	0.5 ± 0.01	11.5 ± 0.2	23.8 ± 0.3	78.1 ± 4.8	0.6 ± 0.39	5.7 ± 0.7	10.1 ± 1.7
Rhododendron	35.4 ± 0.6	1.5 ± 0.02	15.3 ± 0.3	10.3 ± 0.3	86.8 ± 4.9	0.6 ± 0.03	30.7 ± 1.4	52.2 ± 3.7
Vaccinnium	59.1 ± 1.0	0.9 ± 0.02	9.3 ± 0.3	10.9 ± 0.5	68.9 ± 2.2	0.7 ± 0.02	35.3 ± 0.5	48.3 ± 2.0

Table 2. The impact of leaf and root litter composition (monocultures) on three measures of decomposition (mass loss, respiration and exoenzyme activity) after 1 winter, 1 year and 2 years: ANOVA summary results. Enzyme activity is the standardized response (by maximum value within substrate) of enzyme activity averaged across all substrates responses to the model; significant results by substrate are in Appendix S2. Bolded terms indicate significance at $p<0.05$.

		Mass Loss		Respiration		Enzyme Activity	
Source	df	F	p	F	p	F	p
Leaves							
Post-Winter	3,8	**71.2**	**<0.001**	**9.21**	**0.006**	1.68	0.247
1 Year	3,8	**11.91**	**<0.001**	**6.31**	**0.017**	3.75	0.060
2 Years	3,8	**46.04**	**<0.001**	3.75	0.060		
Roots							
Post-Winter	3,8	3.88	0.056	0.62	0.622	**6.70**	**0.014**
1 Year	3,8	**8.59**	**0.007**	3.00	0.095	**17.5**	**<0.001**
2 Years	3,8	3.05	0.092	3.76	0.059		

Table 3. Percent change in litter N pools during decomposition: ANOVA summary of leaf (by species) and root effects. Models included all species combinations which contain the indicated species (i.e., Eriophorum, Ledum and Vaccinium are monocultures and monocultures + Betula, Betula includes monocultures + all 3 species mixture treatments), or all 7 species combinations for roots.

			Change in N	
Species	Sampling	df	F	p
Betula leaves	Post-Winter	3,8	1.49	0.290
	1 Year	3,8	3.56	0.067
	2 Years	3,8	1.09	0.408
Eriophorum leaves	Post-Winter	1,4	3.57	0.132
	1 Year	1,4	4.24	0.109
	2 Years	1,4	5.13	0.086
Rhododendron leaves	Post-Winter	1,4	1.57	0.279
	1 Year	1,4	**8.18**	**0.046**
	2 Years	1,4	2.63	0.180
Vaccinium leaves	Post-Winter	1,4	2.78	0.171
	1 Year	1,4	1.05	0.364
	2 Years	1,4	0.04	0.855
Roots	Post-Winter	6,14	**2.95**	**0.045**
	1 Year	6,14	**5.70**	**0.004**
	2 Years	6,14	0.55	0.762

Figure Legends

Figure 1. The impact of leaf litter composition (monocultures) on three measures of decomposition (mass loss (a-c), respiration (d-f) and standardized exoenzyme activity (g-h)) (mean + SE) over three time periods (after 1 winter (a,d,g), after 1 year (b,e,h) and after 2 years (c,f)). Mass loss is cumulative (calculated from the initiation of the experiment in September 2010) whereas respiration and exoenzyme activity were determined at the endpoint of each time period. Letters indicate significant differences between treatments (Tukey's comparison of means). Tukey's comparisons for enzyme activity (g-h) were analyzed on the average of 5 enzymes.

Figure 2. Seasonal patterns for the proportion of mass remaining for leaf (a) and root (b) litter single species treatments (mean ± SE) overlaid on soil temperatures at 5 cm (leaves) and 10 cm (roots) depth. Eight months decomposing corresponds to 1 winter, and 12 months to after 1 year.

Figure 3. Mixing effects (mean + SE) for mass loss (a-c), respiration (d-f) and exoenzyme activity (g-h) for leaf litter decomposing over three time periods (after 1 winter (a,d,g), 1 year (b,e,h) and 2 years (c,f)). Mixing effects are calculated only for species mixtures. For species mixture treatments "B" refers to *Betula,* "R" to *Rhododendron. "*V" to *Vaccinnium*, and "E" to *Eriophorum*. Asterisks indicate mixing effects are significantly different than zero (t-test, ***=$p<0.001$, **=$p<0.01$, *=$p<0.05$).

Figure 4. Mass loss as proportion decomposed for each species within seven species combinations (mean + SE) for leaf litter decomposing over three time periods (after 1 winter (a), 1 year (b) and 2 years (c), all beginning September 2010). Species were decomposed both alone and in combination: "B" refers to *Betula,* "R" to *Rhododendron. "*V" to *Vaccinnium*, and "E" to

Eriophorum. Lowercase letters above the bars indicate significant differences between species (Tukey's comparison of means, p<0.05).

Figure 5. Change in the proportion of total N lost (negative values) or gained (positive values) when species were decomposed alone and in combination (mean + SE), for leaf litter (a-c) and root litter (d-f) decomposing over three time periods (after 1 winter (a,d), 1 year (b,e) and 2 years (c,f), all beginning September 2010). For leaf litter mixtures (a), black bars represent *Betula* (B) and grey bars the other species in mixture (R=*Rhododendron*, V=*Vaccinnium*, and E=*Eriophorum*); # indicates a significant difference in the N lost/gained between monocultures and the species in mixture, then * beside the bar indicates a significant loss/gain of N within the species treatment. For all other species, * beside the bar indicates a significant N loss/gain averaged across monocultures and mixtures containing that species. For root litter mixtures (d-f), mixtures could not be separated by species after decomposition and each mixture was analyzed as a single treatment (see methods). For both leaves (a-c) and roots (d-f), asterisks indicate N loss/gain is significantly different than zero (t-test, ***=p<0.001, **=p<0.01, *=p<0.05).

Figure 6. Impact of root litter species on three measures of decomposition (mass loss (a-c), respiration (d-f) and standardized exoenzyme activity (g-h)) (mean + SE) over three time periods (after 1 winter (a,d,g), 1 year (b,e,h) and 2 years (c,f)). Mass loss is cumulative (calculated from the initiation of the experiment in September 2010) whereas respiration and exoenzyme activity were determined at the endpoint of each time period. Letters indicate significant differences between treatments (Tukey's comparison of means). Tukey's comparisons for enzyme activity (g-h) were analyzed on the average of 6 enzymes.

Figure 7. Mixing Effects (mean + SE) for mass loss (a-c), respiration (d-f) and exoenzyme activity (g-h) for root litter decomposing over three time periods (after 1 winter (a,d,g), 1 year (b,e,h) and 2 years (c,f)). Mixing effects are calculated only for mixtures. For species mixture treatments "B" refers to *Betula,* "R" to *Rhododendron, "*V" to *Vaccinnium*, and "E" to *Eriophorum*. Asterisks indicate mixing effects are significantly different than zero (t-test, ***=p<0.001, **=p<0.01, *=p<0.05).

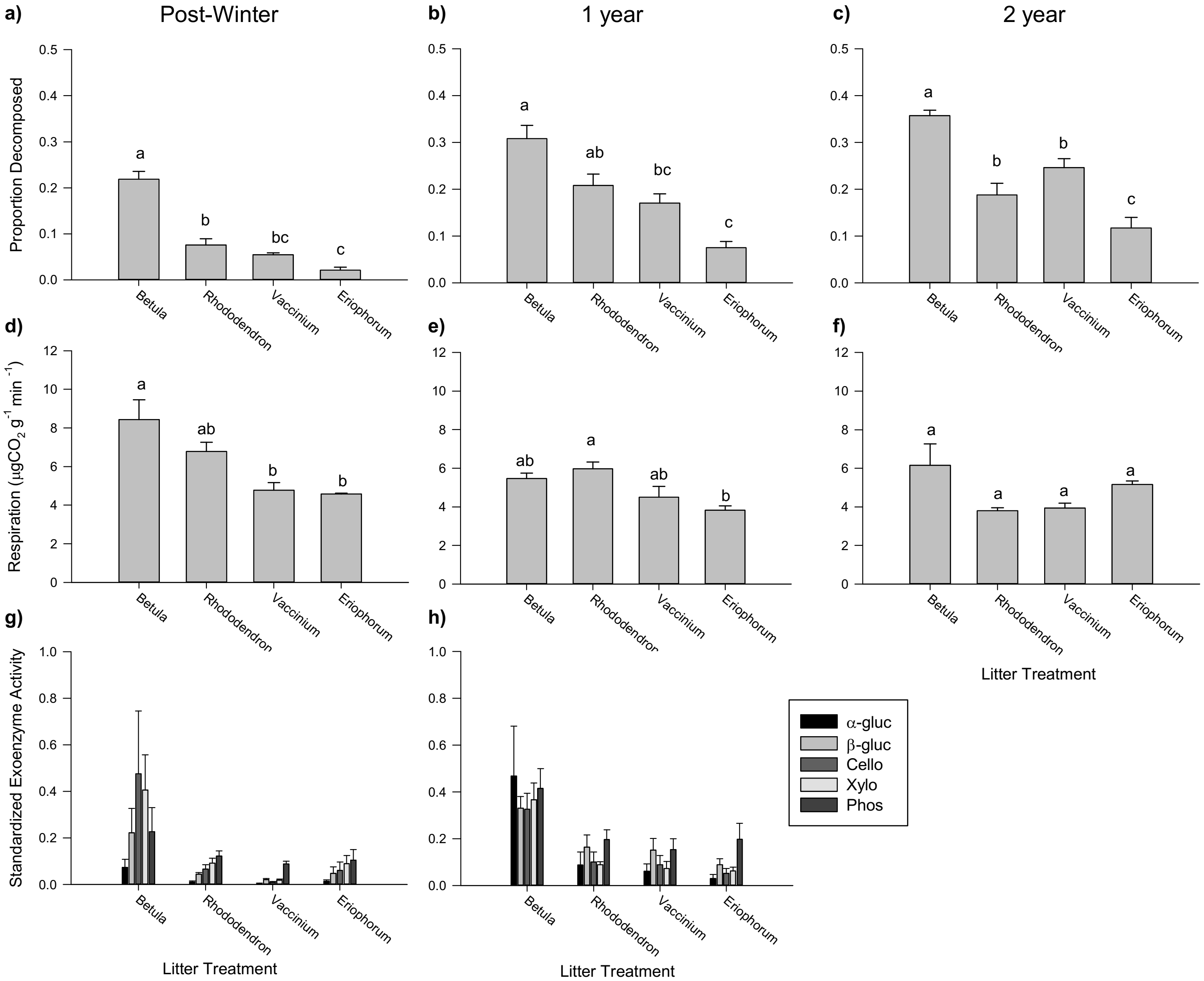
a)
Post-Winter
b)
1 year
c)
2 year
Proportion Decomposed
0.0
0.1
0.2
0.3
0.4
0.5
a
b
bc
c
a
ab
bc
c
a
b
b
c
Betula
Rhododendron
Vaccinium
Eriophorum
d)
e)
f)
Respiration (μgCO2 g-1 min-1)
0
2
4
6
8
10
12
a
ab
b
b
ab
a
ab
b
a
a
a
a
g)
h)
Standardized Exoenzyme Activity
0.0
0.2
0.4
0.6
0.8
1.0
α-gluc
β-gluc
Cello
Xylo
Phos
Litter Treatment

Fig. 1

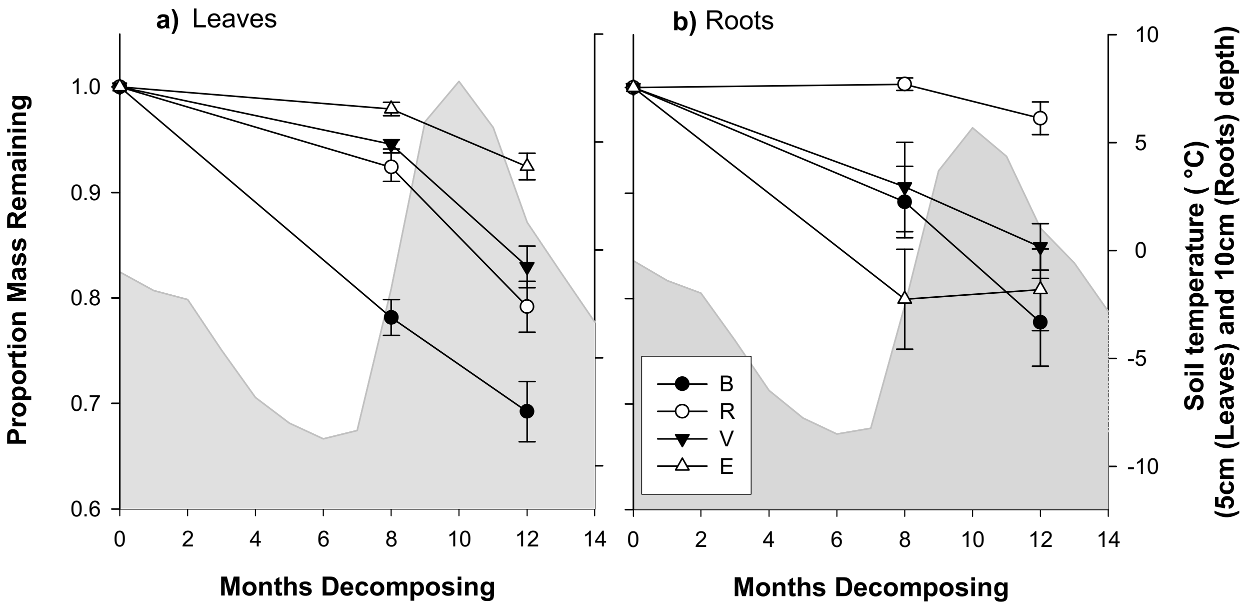
a) Leaves
b) Roots
Proportion Mass Remaining
1.0
0.9
0.8
0.7
0.6
Soil temperature (°C)
(5cm (Leaves) and 10cm (Roots) depth)
10
5
0
-5
-10
0
2
4
6
8
10
12
14
Months Decomposing
B
R
V
E

Fig 2

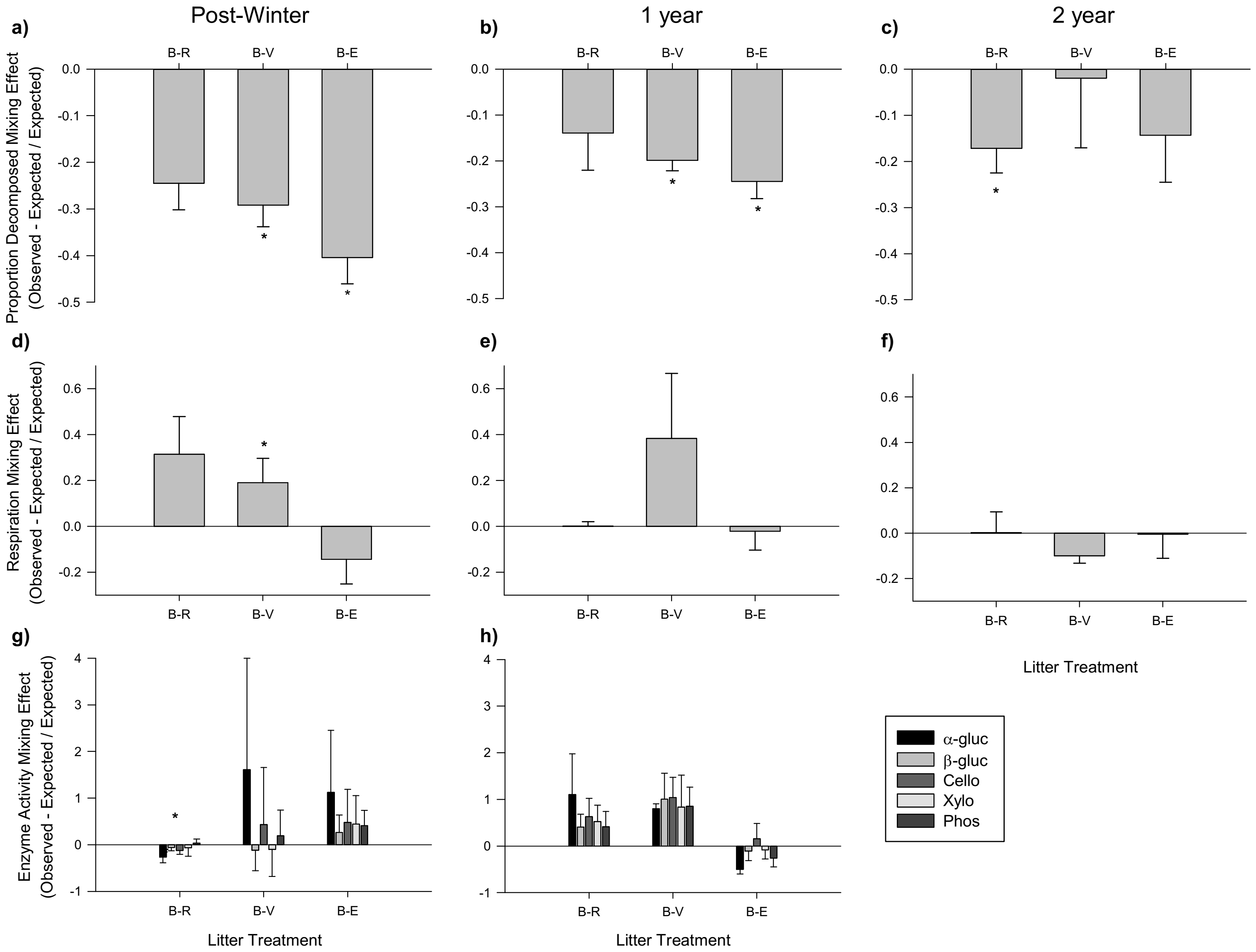
Post-Winter
1 year
2 year
a)
b)
c)
d)
e)
f)
g)
h)
B-R
B-V
B-E
Proportion Decomposed Mixing Effect (Observed - Expected / Expected)
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
*
Respiration Mixing Effect (Observed - Expected / Expected)
0.6
0.4
0.2
0.0
-0.2
Enzyme Activity Mixing Effect (Observed - Expected / Expected)
4
3
2
1
0
-1
Litter Treatment
α-gluc
β-gluc
Cello
Xylo
Phos

Fig 3

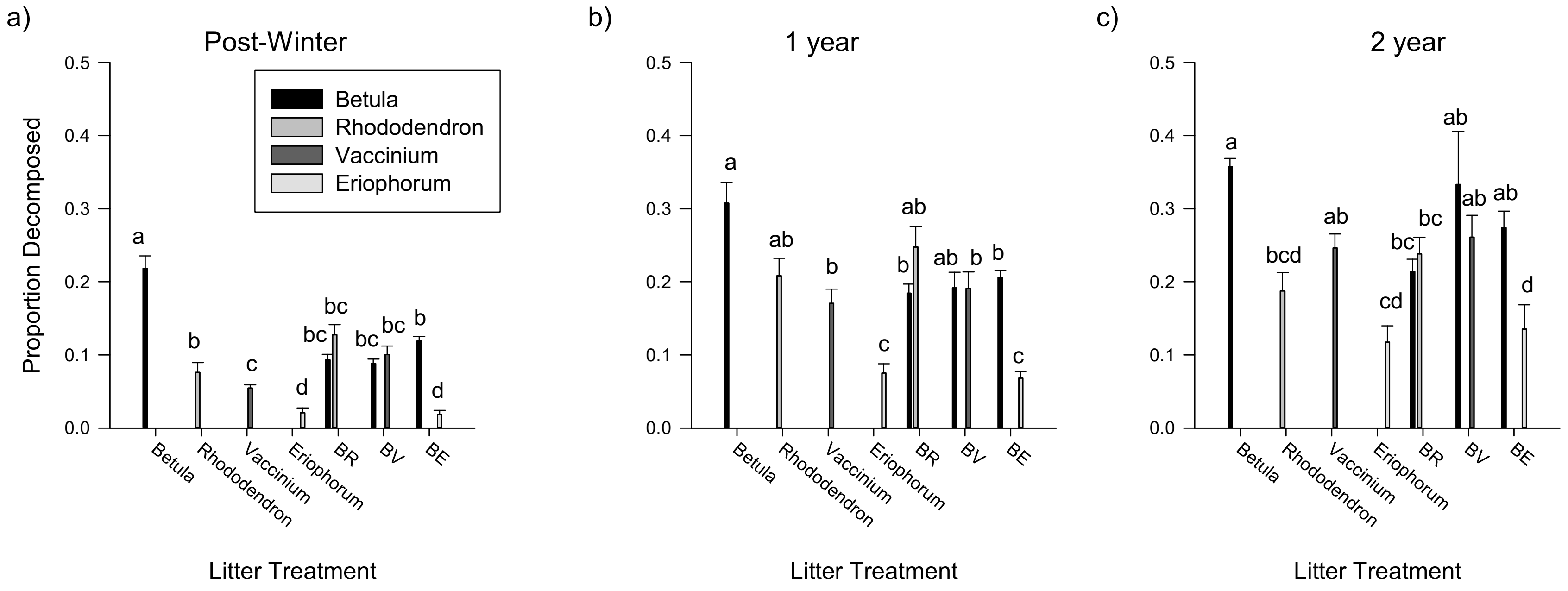
a)
Post-Winter
Proportion Decomposed
0.0
0.1
0.2
0.3
0.4
0.5
Betula
Rhododendron
Vaccinium
Eriophorum
a
b
c
d
bc
bc
bc
bc
b
d
Betula
Rhododendron
Vaccinium
Eriophorum
BR
BV
BE
Litter Treatment
b)
1 year
a
ab
b
c
b
ab
ab
b
b
c
Litter Treatment
c)
2 year
a
bcd
ab
cd
bc
bc
ab
ab
ab
d
Litter Treatment

Fig 4.

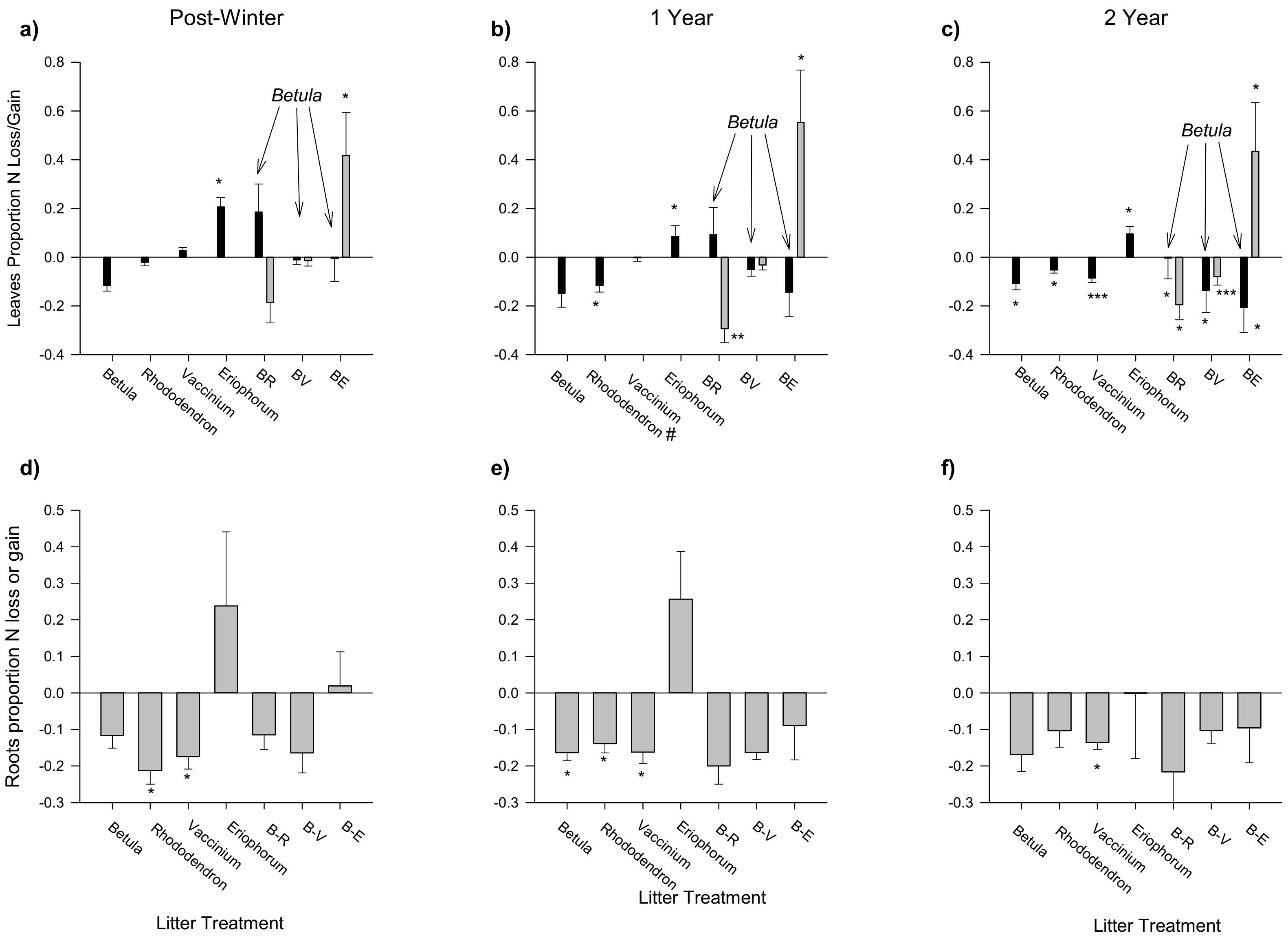
Post-Winter
1 Year
2 Year
a)
b)
c)
d)
e)
f)
Leaves Proportion N Loss/Gain
Roots proportion N loss or gain
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
Betula
Betula
Rhododendron
Vaccinium
Eriophorum
BR
BV
BE
Rhododendron #
B-R
B-V
B-E
*
**
***
Litter Treatment

Fig. 5

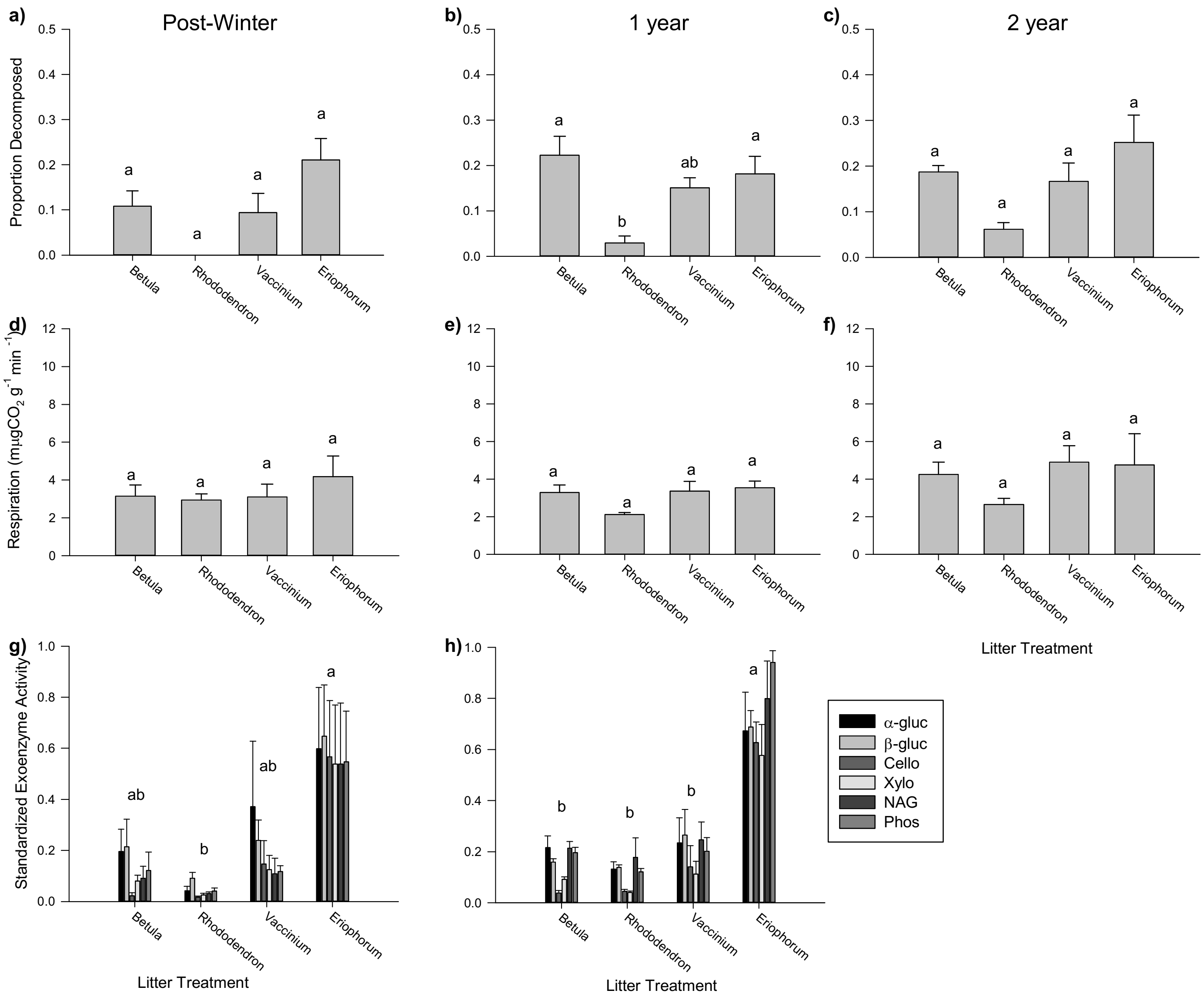
a)
Post-Winter
b)
1 year
c)
2 year
Proportion Decomposed
0.0
0.1
0.2
0.3
0.4
0.5
a
b
ab
Betula
Rhododendron
Vaccinium
Eriophorum
d)
e)
f)
Respiration (mµgCO$_2$ g^{-1} min^{-1})
0
2
4
6
8
10
12
g)
h)
Standardized Exoenzyme Activity
0.0
0.2
0.4
0.6
0.8
1.0
α-gluc
β-gluc
Cello
Xylo
NAG
Phos
Litter Treatment

Fig. 6

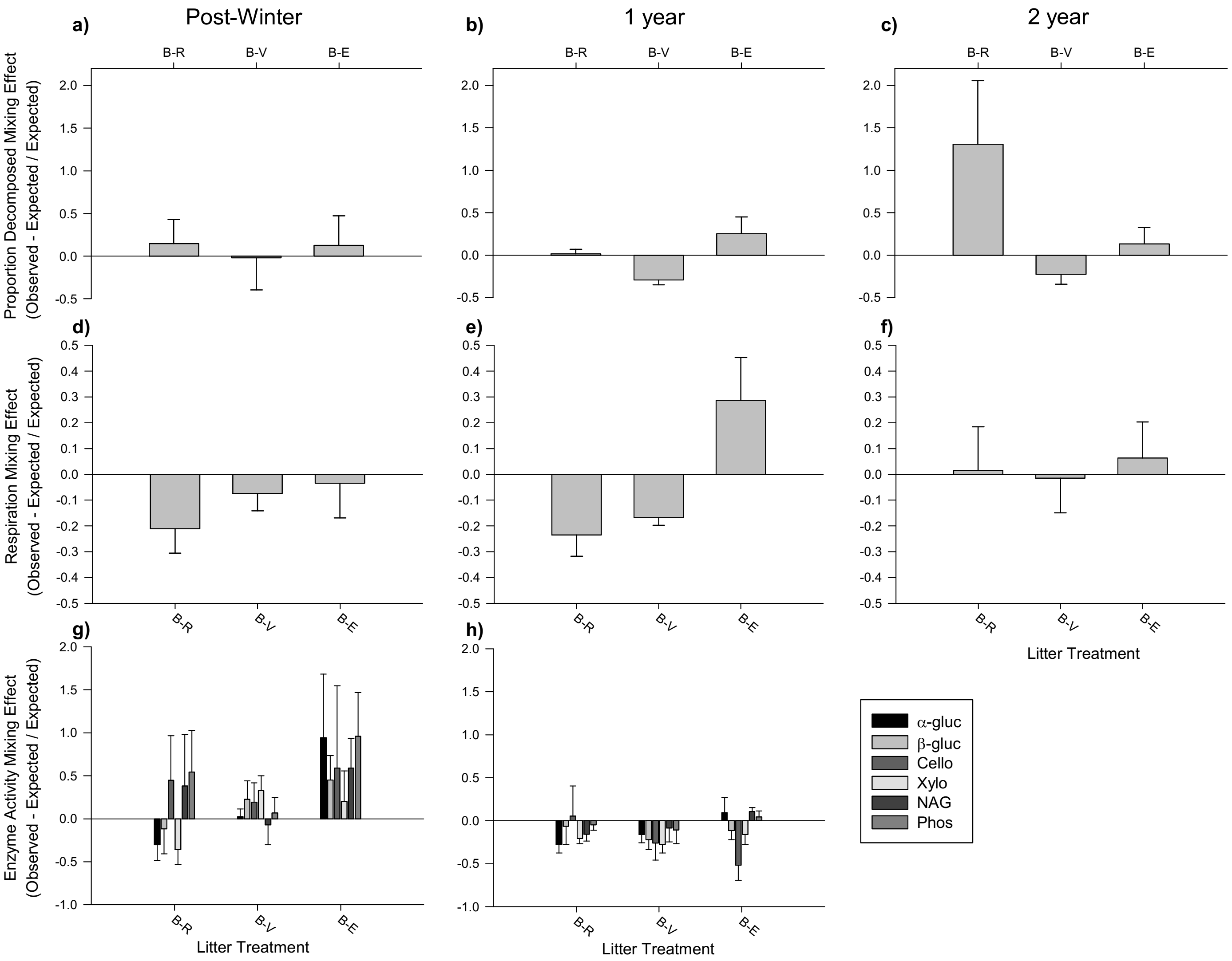
Post-Winter
1 year
2 year
a)
b)
c)
d)
e)
f)
g)
h)
B-R
B-V
B-E
Proportion Decomposed Mixing Effect (Observed - Expected / Expected)
Respiration Mixing Effect (Observed - Expected / Expected)
Enzyme Activity Mixing Effect (Observed - Expected / Expected)
2.0
1.5
1.0
0.5
0.0
-0.5
0.4
0.3
0.2
0.1
-0.1
-0.2
-0.3
-0.4
-1.0
Litter Treatment
α-gluc
β-gluc
Cello
Xylo
NAG
Phos

Fig 7