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Research

Aquatic insect subsidies influence microbial composition and processing of detritus in near-shore subarctic heathland

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Insects are major conduits of resources moving from aquatic to terrestrial systems. While the ecological impacts of insect subsidies are well documented, the underlying mechanisms by which these resources change recipient ecosystems remain poorly understood. Most subsidy inputs enter terrestrial systems as detritus; thus, soil microbes will likely influence the processing of insect subsidies, with implications for plant community composition and net primary productivity (NPP). In a subarctic ecosystem near Lake Mývatn, Iceland where midge (Diptera: Chironomidae) deposition to land is high, we investigated how insect subsidies affected litter processing and microbial communities. We also evaluated how those belowground effects related to changes in inorganic nitrogen, plant composition and NPP. We simulated subsidies by adding midge carcasses to 1-m^2 heathland plots, where we measured effects on decomposition rates and the plant community. We then studied how fertilization treatments (control, KNO_3 and midge-carcass addition) affected graminoid biomass and inorganic nitrogen in greenhouse experiments. Lastly, we conducted a soil-incubation study with a phospholipid fatty acid analysis (PLFA) to examine how midge addition to heathland soils affected microbial respiration, biomass and composition. We found that midge addition to heathland soils increased litter decomposition and graminoid plant cover by 2.6× and 2×, respectively. Greenhouse experiments revealed similar patterns, with midge carcasses increasing graminoid biomass by at least 2× and NH_4^+ concentrations by 7×. Our soil-incubation study found that midge carcasses elevated microbial respiration by 64%, microbial biomass by 43% and shifted microbial functional composition. Our findings indicate that insect subsidies can stimulate soil microbial communities and litter decomposition in subarctic heathlands, leading to increased NPP and changes in plant community composition.

Keywords: decomposition, ecosystem processes, Iceland, insect subsidies, plant composition, soil microbes

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Introduction

Insects are important vectors for energy and nutrient transfer across ecosystem boundaries (Nakano and Murakami 2001, Sabo and Power 2002, Richardson et al. 2010). Thus, insect movement can alter the dynamics of the recipient system, potentially affecting ecosystem productivity (Yang 2004, 2013), community composition (Gratton et al. 2017) and food web structure (Nowlin et al. 2007, Wesner 2010, Recalde et al. 2016). For instance, aquatic insects moving from streams to riparian forests can subsidize diverse communities of terrestrial predators such as spiders (Marczak and Richardson 2007) and birds (Nakano and Murakami 2001), enhancing top–down pressure on herbivores and increasing net primary productivity (NPP) (Henschel et al. 2001). Insect subsidies can also affect plant composition and NPP by increasing soil-nutrient availability (Gratton et al. 2017). While the aboveground impacts of insect subsidies have been documented in many systems (Henschel et al. 2001, Yang 2013, Graf et al. 2017, Gratton et al. 2017), the underlying belowground processes (e.g. decomposition and microbial activity) influencing the responses in NPP and plant composition remain poorly understood.

One way that insects can affect terrestrial ecosystems is through the input of carcasses (Subalusky and Post 2019, Wesner et al. 2019). Insect carcasses are important resource subsidies to terrestrial systems because they are high in carbon and other micronutrients (Yang 2004, Popova et al. 2017). Since microbial communities are often nutrient-limited (Bardgett et al. 2005, Bardgett and Wardle 2010), insect carcasses might stimulate microbial production and activity. Increased microbial production could therefore lead to higher mineralization rates of organic material (i.e. decomposition), potentially increasing the availability of soluble inorganic forms of nutrients for plant uptake. The aboveground responses to insect subsidies observed across ecosystems (Yang 2004, 2013, Gratton et al. 2017) are likely related to the release of nutrients from insect carcasses via increased decomposition and microbial activity. However, research investigating the effects of insect subsidies on the links between litter decomposition, soil microbial communities and aboveground plant responses is scarce.

Most of our existing knowledge on how insect carcasses influence belowground processes and subsequent aboveground plant responses stems from research on periodical cicadas (*Magicicada* spp.) in North America (Karban 1982, Williams et al. 1993, Yang 2004, Nowlin et al. 2008, Yang 2013). For instance, experimentally adding 17-year periodical cicada carcasses to a temperate forest led to increased microbial biomass and nitrogen availability, resulting in higher reproductive rates and growth for forest plants (Yang 2004); however litter or microbial decomposition was not measured. Other terrestrial studies investigating the ecosystem effects of insect subsidies have ignored the responses of microbial communities or decomposition as they relate to plant community changes (Henschel et al. 2001, Gratton et al. 2008, 2017, Garcia et al. 2020). Although previous efforts have increased our understanding of how insect subsidies shape ecosystems via processes occurring belowground, more research investigating their belowground effects across different environmental contexts is necessary. This will help to build a general theory on insect-subsidy impacts, particularly in terrestrial systems that are nutrient-poor.

The Lake Mývatn landscape in northeastern Iceland is a place where insect subsidies are abundant and can potentially affect near-shore subarctic ecosystems (Gratton et al. 2008, Hoekman et al. 2011, Dreyer et al. 2012). At Mývatn, large numbers of midges (Diptera: Chironomidae) emerge from the lake in the summer and form dense mating swarms in the adjacent terrestrial habitat. Mated females return to lay eggs at the lake, but the many, if not most, males perish in the terrestrial environment (Dreyer et al. 2015, Hoekman et al. 2019). While midge subsidies can enter food webs as live prey (Dreyer et al. 2016), most insects evade predation, die and become detritus in the recipient ecosystem (McCary et al. 2021b). Previous research conducted in subarctic heathlands near Mývatn shows that the annual addition of midge carcasses results in significantly more soil inorganic nitrate, plant biomass and a community shift from dwarf shrubs to graminoids (Gratton et al. 2017). However, it remains unresolved how these midge carcasses affect litter decomposition in subarctic ecosystems, which is likely linked to the change in plant composition and biomass observed at Mývatn. Furthermore, little is known about how soil microbes will respond to the addition of the nutrient-rich midge carcasses (i.e. 55% carbon, 9% nitrogen and 1% phosphorus) (Gratton et al. 2008, Gratton and Zanden 2009).

We investigated how insect subsidies influence the processing of detritus and microbial communities in a near-shore heathland in subarctic Iceland. We then examined if changes in these belowground processes correspond to the aboveground responses to insect subsidies observed at Mývatn. We addressed three questions: 1) how do midge inputs to subarctic heathlands affect litter decomposition rates and are those changes linked to plant composition? 2) What are the impacts of midge inputs on graminoid biomass and soil nutrients? 3) How do soil microbial respiration, biomass, composition respond to midge inputs? We performed a series of field, greenhouse and in vitro experiments to address these questions. We predicted that midge inputs to a heathland ecosystem would increase litter decomposition rates, leading to the rapid release of nutrients into the soil environment. The higher decomposition rates will correspond to the enhanced plant biomass, soil nutrients and dominance of fast-growing graminoids documented in previous research (Gratton et al. 2017). We also predicted that the midge carcasses would increase soil microbial activity (i.e. respiration) and biomass, leading to a shift in microbial functional composition.

Material and methods

Study system

To examine how insect subsidies affect recipient heathland ecosystems, we conducted experiments adjacent to Lake Helluvaðstjörn (65°6'N, 17°W), a small lake 6 km from Lake

Mývatn. We selected Helluvaðstjörn because it is an oligotrophic lake with low midge densities (Dreyer et al. 2012); midge deposition within the first 150 m from the shore of Lake Helluvaðstjörn is 0.1% of the eutrophic Mývatn in high-midge years (Gratton et al. 2008). Thus, this site served as a reference for what Mývatn might look like in the absence of midges. Soils in this region are porous, freely drained Andisols with an estimated organic C content of 30 mg g^{-1} (i.e. 3%) (Óskarsson et al. 2004). The ecosystem surrounding Helluvaðstjörn is dominated by dwarf shrubs *Betula nana*, *Arctostaphylos uva-ursi*, *Vaccinium uliginosum*, *Empetrum nigrum*, *Salix lanata* and *S. phylicifolia*. Graminoids (*Deschampsia cespitosa*, *Poa flexuosa*, *Festuca rubra*, *Agrostis stolonifera* and *Eleocharis palustris*) and forbs (*Bartsia alpina*, *Geum rivale* and *Thalictrum alpinum*) are also present but in low numbers (see the Supporting information for a more detailed description of plant communities).

Experiment 1 (field). Effects of midge inputs on litter decomposition and plant composition

We conducted a randomized block design experiment (eight blocks) at Lake Helluvaðstjörn to measure midge impacts on litter decomposition and plant composition (question 1). Within each block, two treatments (no midges added versus midges added) were randomly assigned in 1 × 1-m plots using a random number generator, totaling 16 total plots with eight plots per treatment (Supporting information). Freeze-dried midge larvae (*Chironomus* spp.) were hand-sprinkled evenly into the plots in two 50-g aliquots in 2017 and 2018 (100 g m^{-2} $year^{-1}$) to mimic the high rates of midge deposition at Mývatn (Dreyer et al. 2015). This deposition rate represents the high end of the range for emerging insects from aquatic systems worldwide (0.1 to ~120 g m^{-2} $year^{-1}$; Jackson and Fisher 1986, MacKenzie and Kaster 2004, Gratton and Zanden 2009, Yuen and Dudgeon 2016, Martin-Creuzburg et al. 2017, Stepanian et al. 2020).

Due to extreme fluctuations in midge populations (e.g. midge deposition in the first 50 m from Mývatn can range from 5 to 110 kg ha^{-1} $year^{-1}$; Dreyer et al. 2015), large numbers of adult midges were not available at the time of the study; hence we used commercially available midge larvae as a proxy for Mývatn midges. The larvae are not identical to Mývatn midges, but they are in the same genus as a dominant Mývatn midge species (i.e. *Chironomus islandicus*; Lindegaard and Jónasson 1979), and there is some evidence suggesting that commercial midge larvae and wild-caught adult midges from Mývatn have comparable nutritional contents (i.e. Mývatn midges = 9.2% total N (Gratton et al. 2008); midge larvae = 9.3% total N supplier estimated). Furthermore, because the midge larvae were freeze-dried, the exoskeletal hardness between the larvae and adult midges was similar, meaning they likely had comparable effects on decomposition.

Litter decomposition

We installed litter decomposition bags constructed of 1.7-mm nylon mesh designed to allow access to litter by microbes and arthropods. Plant litter within each bag consisted of a mix of local Icelandic grasses (*Deschampsia*, *Poa* and *Agrostis* spp.). Senesced grass tillers were first dried at 50°C for 48 h, weighed in 2-g aliquots and placed into a litter bag. Litter bags were 8 × 8 cm, with each bag pinned down horizontal to the soil surface. Three litter bags were installed in each plot at the beginning of the experiment (June 2017); we removed one bag on 22 August 2017, 27 June 2018 and finally, 6 August 2018. Following collection, remaining grass tillers within each bag were separated from foreign material (ingrown roots, moss, etc.), dried at 50°C for 48 h, and weighed.

Plant composition

We estimated plant species cover for each experimental plot using a point-intercept sampling method (Gratton et al. 2017) at peak aboveground plant biomass (early August of 2017 and 2018). A 1-m^2 PVC frame with a 10 × 10-cm grid made of nylon string was placed in each plot. At each of the 100 intersections of the grid, we identified all plants to species (except graminoids) and recorded the tallest plant occupying that intersection. Grasses (Poaceae), sedges (Cyperaceae) and rushes (Juncaceae) were categorized as graminoids. The cover of each plant taxon was then calculated for each plot.

Experiment 2 (greenhouse). Midge impacts on graminoid biomass and soil nutrients

We conducted two greenhouse experiments to investigate how midge carcasses affect graminoid biomass and soil nutrients (question 2). In the first greenhouse study (2018), we studied the impacts of freeze-dried midge larvae on graminoid biomass. In the second greenhouse study (2009), we evaluated the effects of adult midge carcasses from Mývatn on graminoid biomass and soil nutrients. We briefly describe the methods of both greenhouse studies here, pointing out key differences between the two studies (see the Supporting information for full details on the greenhouse experiments).

We used a small greenhouse (0.75 × 2.0 × 1 m, L × W × H) to conduct our experiments near Mývatn (200 m from shore). To set up the greenhouse study, soils from the nearshore heathlands of Helluvaðstjörn (< 30 m from experiment 1) were excavated to a depth of ~6 cm. Soils were then bulked into a composite sample and passed through a 1-mm sieve to homogenize and remove large roots. The soil was then added to 8.4 × 8.4-cm diameter pots. For the first greenhouse study, we measured 68 mg (~50 seeds) of a graminoid seed-mix (Turfline Ornamental DFL) obtained from a local horticultural supplier and added them to pots (6 July 2018) with seeds gently buried in the soil to a depth of 1–5 mm. The seed mix consisted of C3 grasses *Festuca rubra* (60%), *Lolium perenne* (35%) and *Poa pratensis* (5%). Here, pots (n = 36) were arranged in a 6 × 6 grid within the greenhouse with treatments assigned randomly; each treatment was replicated 18 times. In the second greenhouse experiment, we added 25 seeds of locally ubiquitous *Deschampsia caespitosa* obtained from a local horticultural supplier (5 June 2009). We used graminoid seeds for the greenhouse experiments because they had the most apparent response to midge addition in

previous experiments (Gratton et al. 2017), and due to the slow growth of heath shrubs in this region, our greenhouse studies were too short to see an observable difference.

In the first greenhouse study, we hand-sprinkled freeze-dried midges in aliquots of 0.72 g pot^{-1} (70.9 cm^2 surface area) to half the plots in a one-time addition, corresponding to the same dose of midges (100 g dry midges m^{-2}) as added in experiment 1. The second greenhouse study had three different nutrient treatments: none, KNO_3 or midges, each replicated nine times and established in a randomized block design (n = 27). Previous research in this system (Gratton et al. 2017) indicated that midge addition increased soil nitrate 36-fold and was correlated with increased graminoid growth in the field. We hypothesized that nitrate was the midge nutrient responsible for increasing plant biomass, and thus, the rationale for adding KNO_3 as a nutrient treatment. All pots were watered on the same day with the same amount of water (50 ml per watering) throughout the experiments (every 2–4 d). Small dishes were placed under each pot to capture excess water during watering and to allow water resorption.

In the second greenhouse study, the midge-addition treatment corresponded to 150 g dry midges m^{-2} $year^{-1}$ or 1.06 g midges pot^{-1} (70.9 cm^2 surface area). This rate was 1.5× higher than in experiment 1 because more accurate calculations (Dreyer et al. 2015) had improved midge deposition estimates since the time of this experiment. Adult midges were collected during peak emergences at Lake Mývatn, brought to the lab and frozen, and dried to a constant mass at 50°C for 3 days. Dried midges were then sprinkled evenly over the surface of the pots in a one-time addition (5 July 2009). On the same day, inorganic nitrogen addition treatments were established consisting of a one-time addition of 0.7 g KNO_3 pot^{-1} (dissolved in 50 ml water), which delivered the same amount of N to pots as the midge-addition treatment. Ion exchange resin bags of 10-g (Rexyn I-300 pellet; Thermo Fisher Scientific) were used to measure available soil inorganic nitrogen leaching from the soils across treatments (Gratton et al. 2017). After the initial additions and watering, and after any excess water had been resorbed by the soil (approximately 1 day later), a small ion-exchange resin bag was placed in the watering dish under each pot where it remained throughout the experiment.

Plant height, biomass and soil nutrient measurements

Forty-five days and sixty days after the initial sowing of seeds in the first and second greenhouse study, respectively, we counted all grass culms in each pot and clipped all the aboveground grass vegetation to the soil level and placed it in a paper bag. Biomass was dried to constant mass at 50°C for 48 h and weighed to the nearest 0.01 mg. For soil nutrients, resin bags were removed and frozen until laboratory extractions were performed following standard procedures (Binkley et al. 1986, Gratton et al. 2017). Resin extracts were measured for nitrate (μg NO_3^- g $resin^{-1}$ day^{-1}) and ammonium (μg NH_4^+ g $resin^{-1}$ day^{-1}) on a flow injection analyzer (Flow Solution 3100; OI Analytical). Additionally, in the first greenhouse study, we measured the height (cm) of the tallest plant in each pot at the end of the study.

Experiment 3 (lab incubation and PLFAs from field plots). Effect of midge inputs on microbial respiration and composition

To test how inputs of midge carcasses affected soil microbial respiration, biomass and composition in heathland soils (question 3), we added midge carcasses to a different set of 1 × 1-m plots from 2008 to 2011 at Helluvaðstjörn (~50 m from shore, Gratton et al. 2017 for details of this long-term addition experiment). Adult midges were collected during peak emergences at Lake Mývatn, brought to the lab and frozen, and dried to a constant mass at 50°C for 3 day. The total mass of midge carcasses added to plots (150 g dry weight midge carcasses m^{-2} $year^{-1}$) represented estimates of high midge deposition near Lake Mývatn (see experiment 2 for the explanation). Midges were added during midge emergences at Mývatn (June, July and August). The midge-addition plots (n = 16) had received yearly carcass additions for four years, corresponding to 'press' treatments in Gratton et al. (2017). Midge-addition plots were compared to the control plots (n = 48).

Microbial respiration

We sampled soil from the experimental plots once in July 2011, 30 days after the last midge addition in the field. We used a small (2.5 diameter × 15 cm) soil corer to collect four cores from each midge-addition and control plot. After each core was taken, we immediately collected the top organic 'duff' layer (typically the 2–3 cm of plant litter and debris above the mineral soil layer). We transported the soil samples to the lab in 160-cc specimen cups (~80 g) and started incubations within 24 h (a standard timeframe). The filled cups remained in a dark lab at 22°C until incubations were initiated.

Using an adapted CO_2 measurement and flux approach of Collier et al. (2014), we placed each specimen cup of soil into a gas collection chamber in the laboratory at 25°C for the incubations. Chambers were 1-l plastic jars with a screw-top lid outfitted with a rubber septum to allow gas sampling with a syringe. Three gas samples were taken with a syringe at 15-min intervals (0, 15 and 30 min) and were injected into an evacuated 5.9-ml glass Exetainter vial. Following the extraction of gas samples, soil cups were moved back into a dark lab at 22°C until the next incubation day (incubations were done on days 1, 3, 5, 10, 17 and 29). All glass vials with the gas samples were stored at −20°C until transport (on dry ice) to the Univ. of Wisconsin-Madison. The samples were processed on an infrared gas analyzer (LI-820 CO_2 Analyzer, LI-Cor) for measurements of CO_2 (Collier et al. 2014). We calculated the rate of CO_2 release for each plot (total new CO_2 30 min^{-1} g $soil^{-1}$) on a given day using an equation described by Collier et al. (2014). We also summed the total CO_2 flux for each specimen cup across all dates to examine cumulative respired CO_2.

Microbial composition

To determine how microbial biomass and composition responded to midge addition, we examined the phospholipid fatty acid (PLFA) profile from the soil organic layer in the

experimental plots (midge-addition and control). We sampled soils as described above and composited four separate soil cores (2.5 diameter × 15 cm) from each plot (n = 16 for midge addition and 48 for control; n = 64 total plots) that were immediately frozen (−20°C). Soils were transported on dry ice to the laboratory at UW-Madison where we freeze-dried the soils and stored them at −20°C until processing. Using a modified PLFA protocol (Oates et al. 2017), we extracted phospholipids from 3 g of freeze-dried soil from each sample using a chloroform–methanol extraction method (see the Supporting information for full details). We used lipid biomarkers to determine broad microbial groups, including fungi, gram-negative bacteria, gram-positive bacteria and Actinobacteria (Zelles 1999, Oates et al. 2017). We then measured the total amount of lipids in each plot (i.e. total microbial biomass g soil^{-1}; see the Supporting information for functional designations).

Statistical analyses

Litter decomposition and soil respiration

To evaluate how midge addition affected litter decomposition, we first calculated the proportion of initial litter mass remaining by dividing the mass at each harvest date by initial plant mass in the litter bags. We then determined decomposition rates (k) by fitting the proportion of litter mass (dw) remaining in each plot over time (months) with a negative exponential decay model (Olson 1963). We used a linear mixed-effects model (LMM) to test the effects of midge addition (no midges added versus midges added) on k; block was treated as a random effect.

We also performed an LMM with two fixed factors (midge treatment and day) to determine midge effects on microbial respiration rates. We included a treatment × day interaction to estimate differences in respiration rates throughout the experiment. Day was $\log_e$ transformed and modeled as a continuous variable since the start of the incubation (i.e. day 1, 3, 5, 10, 17 and 29). The random effects included incubation cup to account for the repeated sampling of cups through time. LMMs were fit using the 'lme4' package in R ver. 4.0.3 (Bates et al. 2015, <www.r-project.org>); Kenward–Roger approximations for degrees of freedom were used to calculate p-values (type III sums of squares) with the 'lmerTest' R package (Kuznetsova et al. 2017).

Plant biomass, height, CO_2 respired, microbial biomass and inorganic nitrogen

We performed Welch's t-tests in R (ver. 4.0.3) to examine midge effects on graminoid height, cumulative CO_2 respired and microbial biomass. All variables were $\log_e$ transformed before analysis.

To examine the effects of nutrient treatments on graminoid biomass, NO_3^-, NH_4^+ and total inorganic nitrogen in experiment 2, we conducted LMMs with nutrient treatment as a fixed factor: control, KNO_3 or midge addition; block was included as a random effect. The LMMs used Kenward–Roger approximations for degrees of freedom and were fit using the 'lme4' and 'lmerTest' packages in R (Bates et al. 2015, <www.r-project.org>).

Plant and microbial composition

Plant community responses to midge addition were evaluated using permutational analysis of variance (PERMANOVA: 10 000 permutations; type III SS) (Anderson et al. 2008). The PERMANOVA had two fixed factors and their interaction: midge addition (no midges added versus midge addition) and year (2017 and 2018). Block was included as a random effect to account for spatial variation (Anderson et al. 2008). Before the analysis, we scaled the data by centering the cover of each plant species to a mean of zero and dividing by the standard deviation; species < 0.05% of total plant cover were removed. Using this z-score transformed data, we calculated a Euclidean dissimilarity distance matrix between plots. We conducted a principal coordinates analysis (PCO) to visualize differences in plant composition in two-dimensional space. Vector overlays were used to show which plant taxa were associated with each treatment; a vector reflects partial correlation coefficients for a species against the two axes (we only show vectors with $R^2 > 0.45$ for clarity).

To assess microbial composition responses to midge addition, we first calculated relative microbial abundance by converting each taxon to mol% (removing any value < 0.05%) and dividing by total abundance for that sample. We then conducted a PERMANOVA (10 000 permutations; z-score transformed; Euclidean dissimilarity, type III SS) to evaluate differences in midge treatment (control versus midge addition). We also used parallel permutational analyses of multivariate dispersions (PERMDISP; 10 000 permutations) to evaluate if there were differences in group variability or centroids among treatments. We used a PCO to visualize differences in microbial composition. Vector overlays are partial correlation coefficients for microbial taxon against the two axes (vectors represent $R^2 > 0.3$). PERMANOVAs, PERMDISP and PCOs were performed using PRIMER-E/PERMANOVA + software (Anderson et al. 2008).

Results

Experiment 1 (field). Effects of midge inputs on litter decomposition and plant composition

Litter decomposition

The litter bag assay indicated that plant litter in the midge-addition plots decomposed 2.6× faster over 15 months than plant litter in control plots (decay constant k, LMM; $F_{1,7} = 10.50$, $p = 0.014$, Fig. 1, Supporting information).

Plant composition

Plant composition showed an interaction between midge addition and year (PERMANOVA, Pseudo-$F_{1,7} = 4.22$, $p = 0.01$, Supporting information); thus, we analyzed

plant composition independently according to year. At the end of the first year (August 2017), plant composition did not differ according to midge addition (PERMANOVA; Pseudo-$F_{1,7}=0.72$, $p=0.62$). However, by the end of the 15-month experiment (August 2018), plant composition had shifted in the midge-addition plots (PERMANOVA; Pseudo-$F_{1,7}=2.11$, $p=0.04$), with the difference in plant composition being attributed to the increase in graminoid cover (Fig. 2). This result is highlighted in the PCO ordination where the midge-addition treatments diverged from the no-midge (control) plots (Fig. 2b, PCO axis 2). In contrast, the dwarf shrubs *B. nana* and *A. uva-ursi* were consistently more associated with the no-midge treatment plots (Fig. 2b) in the PCO ordination for 2018. Graminoids in the midge-addition plots only represented ~25% of mean plant cover in 2017 (Supporting information); this proportion had increased to ~44% in 2018 (Supporting information for full details on plant species cover).

Experiment 2 (greenhouse). Midge impacts on graminoid biomass and soil nutrients

Plant height and biomass

For the first greenhouse study, the addition of midges to pots increased both plant biomass (+92%; $t=-5.56$, $p < 0.001$) and height (+62%; $t=-4.80$, $p < 0.001$) relative to the no-midge treatments (Fig. 3). In the second study, the midge-addition plots had 12× and 9× more biomass than the control and KNO_3 treatments, respectively (LMM; $F_{2,16}=79.21$, $p < 0.001$, Fig. 4a, Supporting information).

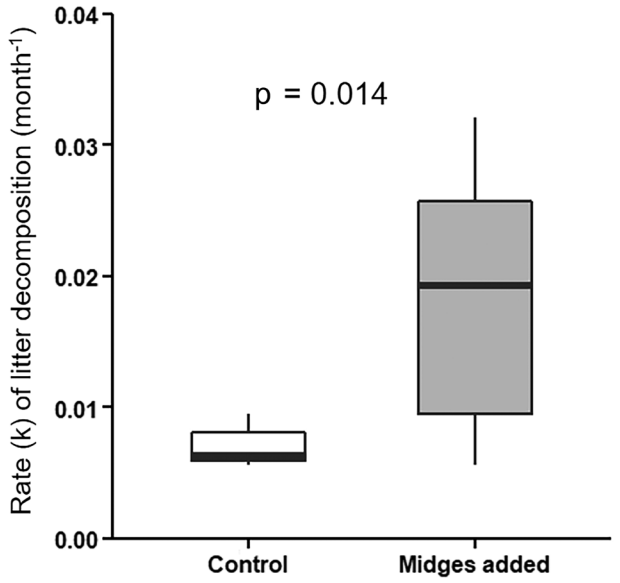

Figure 1. The effects of midge addition on litter decomposition rate (*k*) in Experiment 1, which was calculated from the start to the end of the experiment (15 months). p-values were calculated from linear mixed-effects models (LMMs). The top and bottom of the boxes indicate the first and third quartiles, with the center line denoting the median. The whiskers show 1.5 times the interquartile range.

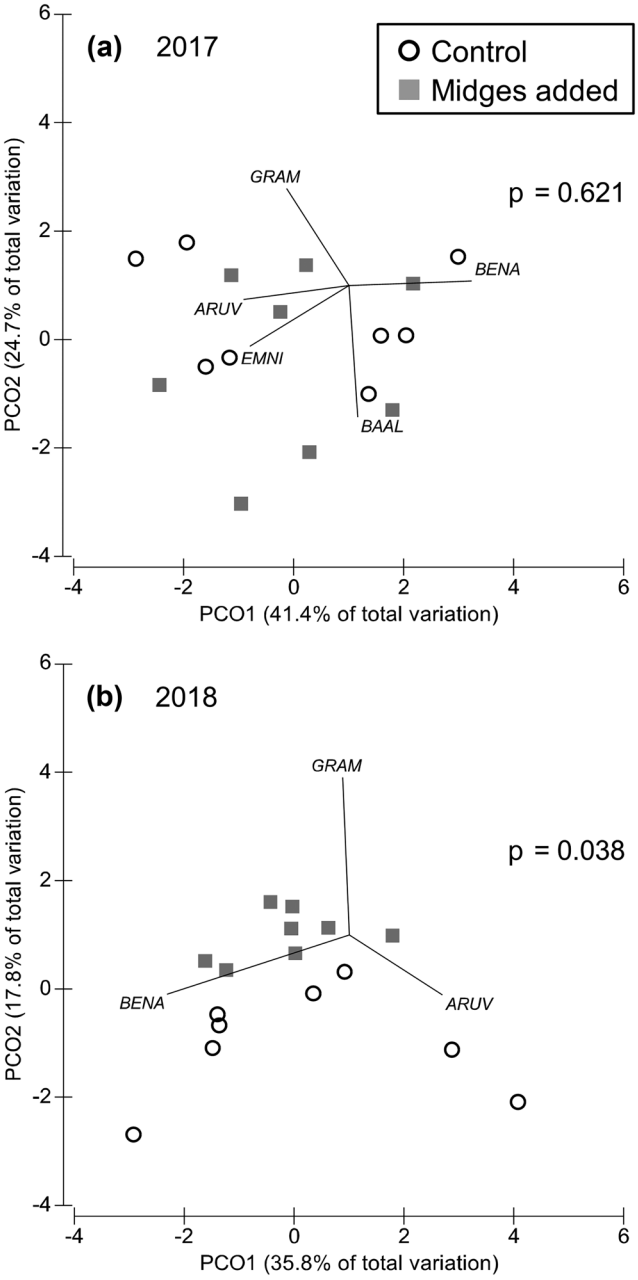

Figure 2. Principal coordinates analysis (PCO) plots to show the changes in plant composition measured at peak biomass (August) as a function of midge addition in (a) 2017 and (b) 2018. PCO biplots of z-score transformed plant composition data are based on a Euclidean dissimilarity matrix. Each symbol on the ordination plot represents communities for one of the 16 experimental plots in that year of the experiment. The direction and length of vector overlays indicate the strength of the association (multiple partial correlation coefficient) between the ordination axes and the associated labeled taxon, showing only vectors with $R^2 > 0.45$. Most species were present in both years, but their relationship with midge addition might have differed in 2017 and 2018. ARUV = *Arctostaphylos uva-ursi*; BAAL = *Bartsia alpina*; BENA = *Betula nana*; EMNI = *Empetrum nigrum*; GRAM = graminoid functional group. Data are from experiment 1.

Inorganic nitrogen

Exchangeable NO_3^- concentrations from resin bags were highest in the KNO_3 treatment (LMM; $F_{2,16}=16.32$, $p < 0.001$, Fig. 4b, Supporting information), being 6× and 3× higher than the control and midge-addition treatments, respectively. This trend was different for exchangeable NH_4^+ concentrations ($F_{2,16}=6.39$, $p=0.009$, Fig. 4c, Supporting information), which exhibited the highest values in the midge-addition treatment. Concentrations of NH_4^+ in the midge-addition treatment were 7× higher than the KNO_3 treatment and 14× higher than the control plots (Fig. 4c). Following the overall pattern of NO_3^- concentrations, total inorganic N was highest in the KNO_3 treatment ($F_{2,16}=14.90$, $p < 0.001$, Fig. 4d, Supporting information), which was 6× and 2× higher than the control and midge-addition treatments, respectively.

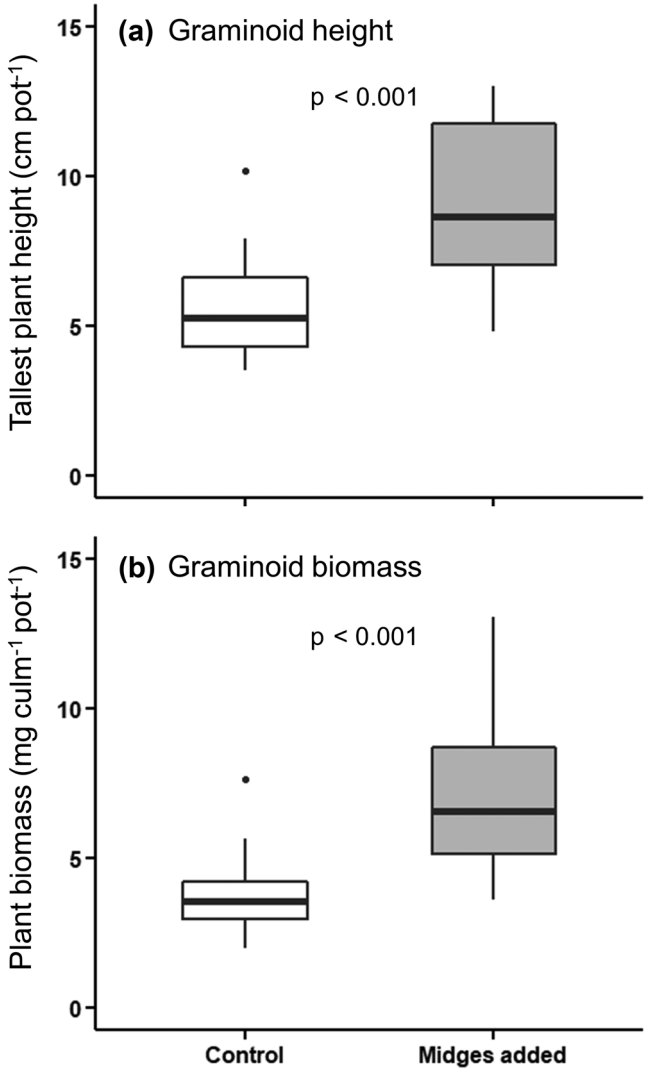

Figure 3. Effects of midge addition on (a) plant height (cm) and (b) plant biomass (mg DW per culm) in the first greenhouse study in experiment 2 (45 days post sowing). The top and bottom of the boxes indicate the first and third quartiles, with the center line denoting the median. The whiskers show 1.5 times the interquartile range.

Experiment 3 (lab incubation and PLFAs from field plots). Effect of midge inputs on microbial respiration and composition

Microbial respiration

Respiration rates over time were higher in the midge-addition treatment compared to the control plots (LMM; $F_{1,169}=12.76$, $p < 0.001$, Table 1), with a 64% increase in respiration in the former (control = 15.1 rate of respired CO_2 [day^{-1}]; midge addition = 24.7). Total respired CO_2 was also different from the control and midge-addition plots throughout the 29 days of the soil-incubation experiment (Welch's t-test; $t_{1,22}=-5.05$, $p < 0.001$, Fig. 5). On average, the midge-addition treatments produced 63% more CO_2 than the control soils throughout the 29-day incubation experiment.

Microbial composition

Midge addition to heathland plots increased total microbial biomass by 43% compared to the control plots (Welch's t-test; $t_{1,20}=2.65$, $p=0.02$). Soil microbial composition also differed between the control and midge-addition treatments (PERMANOVA; Pseudo-$F_{1,61}=3.43$, $p=0.001$, Fig. 6). Differences in group variability (dispersion) did not contribute to the divergence in microbial composition (PERMDISP; $F_{1,61}=0.02$, $p=0.90$), indicating group centroids of the midge-addition plots were different from the control plots. The treatment effect is illustrated in a PCO ordination of the lipid profiles of the different experimental field plots, which shows the midge-addition treatments clustered on the left side of PCO axis 1, whereas the control treatments are generally correlated with the right half of PCO axis 1. This result suggests that the midge treatment supported a different microbial community than the control, which primarily stemmed from differences in the abundance of several functional groups of bacteria (i.e. 15:0 ISO, 15:0 ANTEISO, 16.00, 16:1 ω7c, Fig. 6).

Discussion

Ecological theory holds that spatial subsidies can have important effects on ecosystem structure and functioning (Polis et al. 1997, Leroux and Loreau 2012, McCary et al. 2021a). However, the magnitude and direction of ecosystem responses to allochthonous inputs are highly variable (Marczak et al. 2007, Allen and Wesner 2016), with limited knowledge of the underlying belowground processes influencing the aboveground responses to subsidies. Because the activity of microbial communities and the processing of detritus are intimately linked to NPP and plant composition (Bardgett and Wardle 2010), subsidy impacts on belowground processes likely mediate the responses observed aboveground. We found that adding insect carcasses – an external resource subsidy – to a subarctic heathland increased litter decomposition rates and microbial respiration. These belowground

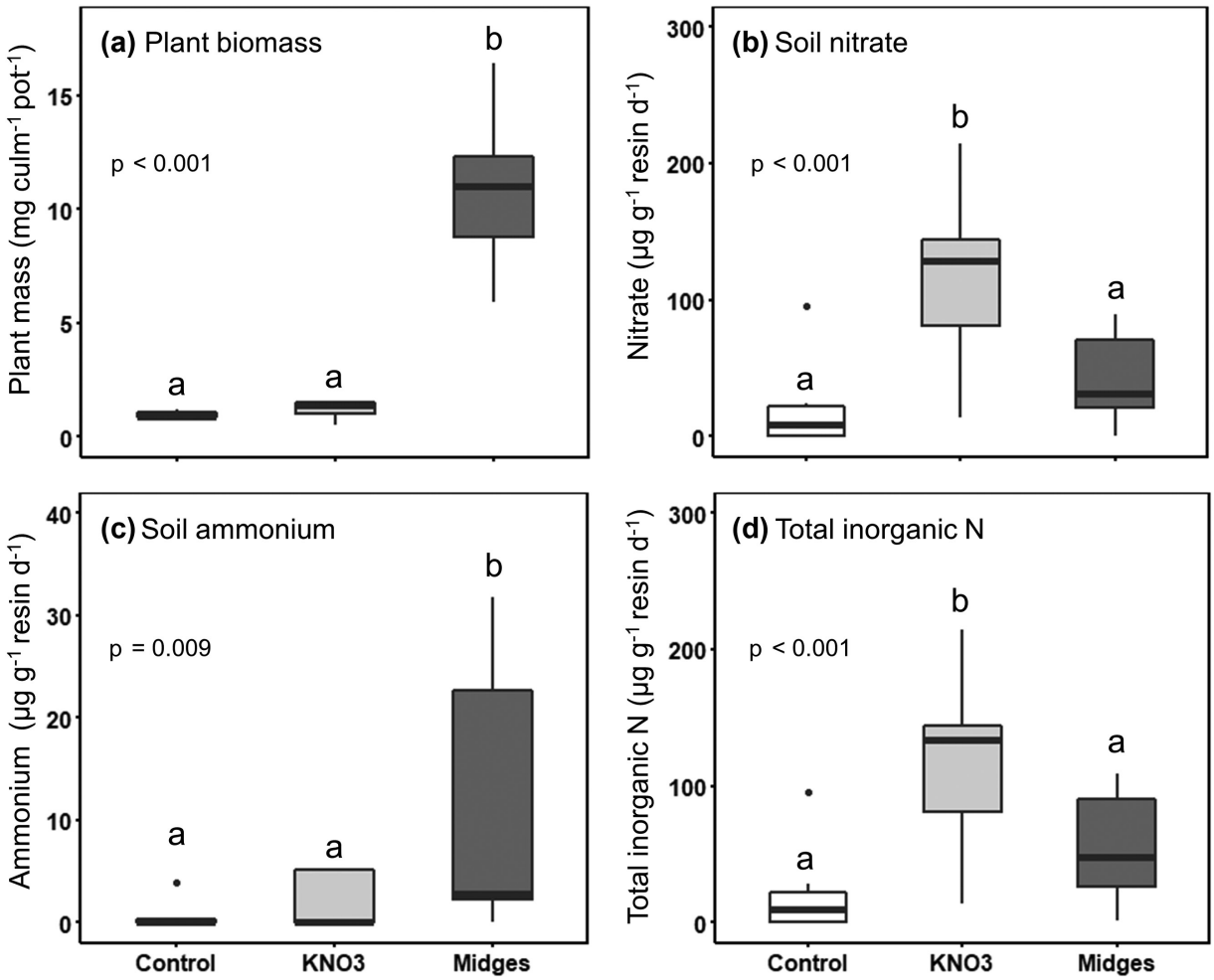

Figure 4. (a) Final graminoid biomass (mg DW per culm) from the second greenhouse study in experiment 2 from pots that were fertilized with an aqueous solution of KNO_3, dry midges or left as controls. The effects of fertilization treatment on soil (b) nitrate, (c) ammonium and (d) total inorganic nitrogen (N) in the second greenhouse study. Soil nutrients were collected from ion exchange resins placed under pots. Midge carcasses and inorganic N additions were made so that the total N delivered to plants was the same. For all boxplots, the top and bottom of the boxes indicate the first and third quartiles, with the center line denoting the median; the whiskers show 1.5 times the interquartile range.

processes were also associated with higher graminoid biomass, ammonium concentrations and a plant community shift from dwarf shrubs to fast-growing graminoids. These findings indicate that insect subsidies can stimulate belowground processes, resulting in increased NPP and changes in plant community composition in subarctic ecosystems.

Low nutrients and slow growth generally characterize subarctic heathlands in Iceland because of the cold and dry climate (Arnalds et al. 1987). Hence, plant communities in this region are dominated by dwarf shrubs, with graminoids present at low frequencies. However, when we added midge carcasses to this system, we found rapid growth of graminoids

Table 1. The results from the linear mixed-effects model testing for the main and interactive effects of midge addition on microbial respiration rates (decomposition) in experiment 3. Marginal R^2 denotes the amount of variance explained by the fixed effects, whereas the Conditional R^2 represents the amount of variance explained by both the fixed and random effects. The results are from type III F-tests.

Factor	Numerator df	Residual df	F-value	p	Marginal R^2
Treatment	1	195.25	287.24	< 0.001	0.593
Log(day)	1	168.97	32.02	< 0.001	
Treatment × Log(day)	1	168.97	12.76	< 0.001	

Random effects	Variance	Conditional R^2
Plot	4783	0.666
Residual	22 003	

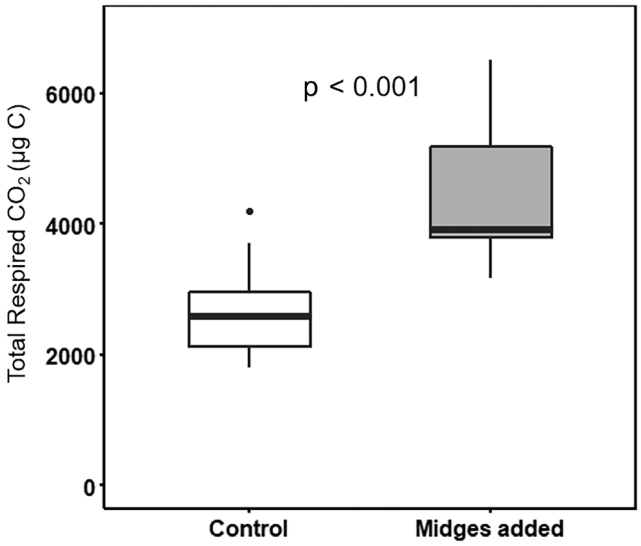

Figure 5. Box plots showing the effect of midge addition on total CO_2 respired over the 29 days of soil incubations in experiment 3.

relative to the slower-growing heath vegetation, leading to a community shift from dwarf shrubs to graminoids after just two years of midge addition. Our manipulative greenhouse experiment confirmed that midge carcasses increase graminoid biomass, which is also associated with higher levels of nutrients in the soil. Other studies have found similar results

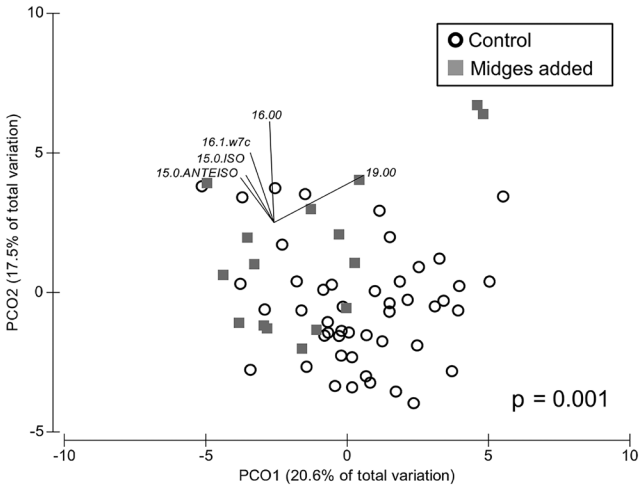

Figure 6. Principal coordinates analysis (PCO) plots showing differences in soil microbial composition as a function of midge addition in experiment 3. Bi-plots of microbial data are based on a Euclidean dissimilarity matrix (z-score transformed). Each symbol on an ordination plot represents a given microbial community for the 63 plots sampled in 2011 (1 plot removed due to low lipid extraction); the length and direction of vector overlays indicate the strength of the association (multiple partial correlation coefficient) between the ordination axes and the associated labeled taxon, showing correlations with $R^2 \geq 0.3$. The vector overlays represent five of the most highly correlated microbial functional groups. 15:0 ANTEISO = gram-negative bacteria, 15:0 ISO = gram-positive bacteria, 16:00 = unspecified bacteria, 16:1 ω7c = gram-negative bacteria and 19:00 = unspecified bacteria.

that arctic ecosystems are sensitive to nutrient inputs, with significant consequences for plant composition and productivity (Shaver et al. 2001, Gough et al. 2002). For example, inorganic nutrient addition shifted the recipient plant community composition in a wet arctic tundra towards woody vegetation, causing a 2.5× increase in NPP following several years of experimental manipulation (Shaver et al. 2001, Gough et al. 2002).

We found that midge addition altered the processing of detritus by increasing litter decomposition rates. Previous studies in terrestrial ecosystems have yet to document subsidy impacts on litter decomposition, but our research indicates that insect subsidies can significantly accelerate nutrient cycling. While the mechanisms by which midge carcasses increased decomposition rates are unclear in our study, stimulated soil microbial activity was likely responsible for the high rates of litter disappearance (Pray et al. 2009). Soil microbes are mostly carbon-limited (Demoling et al. 2007, Kolb et al. 2009), and because midge carbon is easily accessible (i.e. low C:N ratios [~5:1]), soil microbial respiration and biomass increased. For instance, soil microbes respired 64% more CO_2 in treatments with midge carcasses than the control indicating increased activity. Furthermore, the byproduct of carbon breakdown by soil microbes is the mineralization of organic nitrogen. Ammonium concentrations in the midge-addition plots were > 7× higher than the control and KNO_3 treatments in experiment 2, suggesting that soil microbes responded to the enriched carbon inputs by making more nitrogen available for plant uptake. These results are also similar to the effects of periodical cicadas on belowground nutrients leading to increased microbial biomass and subsequent plant productivity (Yang 2004, 2013).

It is worth noting that the KNO_3 addition in the greenhouse experiment did not appear to affect plant biomass compared to the control. In a previous experiment (Gratton et al. 2017), midge addition increased soil nitrate 36-fold compared to background levels, suggesting that midges impacted plant biomass via increased nitrate. However, our greenhouse study indicates this was not the case. We propose several hypotheses to explain why the different treatments (i.e. no midges, KNO_3 and midge carcasses) had different effects on graminoid biomass. First, despite finding a positive association between ammonium concentrations and plant biomass in the midge-addition treatment, it is possible that other midge nutrients caused the enhanced plant growth. Besides being about 9.2% nitrogen content, midge carcasses also consist of carbon (~55%), phosphorus (~1%) and other micronutrients (Gratton et al. 2008). Furthermore, the other nutrients in the midge carcasses probably stimulated higher microbial activity, resulting in more plant-available nutrients compared to the no-midge and KNO_3 treatments. The stoichiometric ratios of C:N:P were also different across treatments, which might also explain the differences between nutrient treatments.

Our experiments found that midge addition consistently increased microbial biomass and respiration rates. We also revealed that midge addition led to differences in microbial

lipid composition, causing a shift in bacteria functional biomarkers (Fig. 6). The higher microbial biomass for multiple microbial biomarkers (Supporting information), respiration rates and composition shift could explain the increased availability of plant-available nitrogen that we found in our experiments. For example, organic fertilizers with low C:N ratios (i.e. white clover *Trifolium repens* litter) in a lab-incubation experiment resulted in 2.5× higher mineralization rates of total nitrogen compared to the control, corresponding to increased numbers of bacteria (gram-positive and gram-negative), Actinomycetes and fungi (Masunga et al. 2016). The totality of these findings suggests that insect subsidies can increase soil microbial production (i.e. biomass and respiration), thereby mediating the aboveground plant response to allochthonous inputs.

Though we used a combination of midge larvae and adult carcasses in our field, greenhouse and in vitro experiments, our data indicate that these two life stages have similar effects on Mývatn's terrestrial ecosystem. We found a shift from a heath-dominated (e.g. dwarf shrubs) to a graminoid-dominated (forbs, sedges and grasses) plant community when midge larvae were added, consistent with previous research that used Mývatn adult midges (Gratton et al. 2017). Furthermore, we observed faster litter decomposition in the field (experiment 1) when using midge larvae, which is also consistent with the increased microbial respiration rates recorded in our soil-incubation study that used adult carcasses (experiment 3). Lastly, we recorded plant biomass increases regardless of adding midge larvae or adults in the greenhouse studies. While we acknowledge that there might be inherent morphological, physiological or nutritional differences in the midge larvae and adult carcasses used in this study, their positive impacts on plant composition, biomass and decomposition suggest that they have comparable effects on this subarctic ecosystem.

Conclusions

In arctic ecosystems, where nutrient availability often limits primary production, resource subsidies can have profound ecosystem-level effects (Gratton et al. 2017). We show that the addition of insect carcasses to subarctic heathland soils elevated litter decomposition rates and altered microbial composition and activity, ammonium concentrations, leading to increased plant biomass and a shift from dwarf shrubs to herbaceous graminoids. Hence, aboveground responses to insect subsidies are likely mediated by soil microbial production and decomposition. Our findings indicate the significance of insect subsidies in maintaining subarctic ecosystem structure and functioning via belowground processes.

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Author contributions

Matthew A. McCary: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Resources (lead); Software (lead); Supervision (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Madeline D. Kasprzak**: Conceptualization (supporting); Data curation (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Validation (supporting). **Jamieson C. Botsch**: Data curation (supporting); Investigation (supporting); Methodology (supporting); Writing – review and editing (supporting). **David Hoekman**: Conceptualization (supporting); Data curation (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Writing – review and editing (supporting). **Randall D. Jackson**: Conceptualization (supporting); Data curation (supporting); Funding acquisition (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Resources (supporting); Software (supporting); Supervision (supporting); Writing – review and editing (supporting). **Claudio Gratton**: Conceptualization (equal); Funding acquisition (supporting); Investigation (supporting); Methodology (equal); Project administration (supporting); Resources (supporting); Writing – review and editing (supporting).

Data accessability

All data and accompanying scripts are available on Zenodo: <https://doi.org/10.5281/zenodo.4903950>.

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