MIDGE CARCASSES ALTER THE STRUCTURE AND FUNCTION OF PLANT AND SOIL COMMUNITIES IN ICELANDIC HEATHLANDS

by

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GENERAL ABSTRACT

Historically, terrestrial ecosystems have been viewed as providers of nutrients to lakes and rivers through such processes as runoff and litter inputs from the near-shore vegetation. However, recent evidence supports a more reciprocal relationship, where lakes and streams provide nutrients to the near-shore terrestrial systems. One way in which bodies of water can influence land is through the movement of insects across the aquatic-terrestrial boundary. Insects that emerge from lakes and streams are rich sources of carbon and nitrogen and can affect the near-shore food-web by acting as food for predators on land. These near shore communities may also be affected by changes in nutrient availability from insect carcass death on land following emergence. I hypothesized that insect carcass deposition to land following an emergence event will facilitate changes in near-shore plant and soil community composition and function.

In Northeast Iceland, lakes support varying levels of emergent insects (midges) and are surrounded by nutrient limited plant communities consisting of grasses, forbs, woody shrubs, and low-laying heathland plants. As midges are high in nitrogen (~10%), I expected plant communities to be dominated by plants best able to use nutrients quickly (i.e., grasses) in places where midges were most abundant. In a broad scale survey, I found that plant communities increased grass dominance in areas receiving high levels of midge inputs. I hypothesized that these shifts in plant communities were likely driven by changes in soil nutrients following midge deposition to land.

To directly measure the effects of midge carcass deposition on soil communities, we set up a manipulative experiment near a low-midge lake and hand added carcasses to small plots. Midge carcasses were added for multiple years (press) or at a single time point (pulse)

to examine if soil communities changed with prolonged inputs compared to episodic inputs of midges. I expected that the consistent midge addition in the "press" treatment would increase decomposition rates and support different soil microbial communities than a pulsed treatment due to reductions in nutrient limitation that would accrue over time. I found that decomposition rates were significantly higher in plots receiving pressed carcass additions, compared to either pulse or control plots to which no midges had been added. In addition, microbial communities were different compared to control and pulsed treatments. I also found shifts microbial community structure in the pulsed treatment compared to the control and pressed treatment, suggesting that a single nutrient addition altered soil communities, but this effect disappeared one year after the initial pulse of midges. The direct changes in soil communities brought about by midge deposition to the soil may ultimately explain the changes measured in plant communities across the landscape.

Movement of insects from aquatic to terrestrial systems changed near-shore communities through alterations of soil microbial communities and nutrient cycling. The effects seen at lakes in Northern Iceland may represent a general phenomenon for any lake or stream, although perhaps not at the same magnitude as seen in Iceland where midge emergences were extreme. Insect deposition to land may change microbial communities and associated ecosystem cycling rates of C and N, with potential effects on plant communities in any near-shore ecosystem.

Chapter 1. Aquatic insect deposition to land promotes grass dominance in a heathland ecosystem

ABSTRACT

Insect emergence from lakes can represent a significant input of carbon and nitrogen (N) to adjacent terrestrial communities, affecting ecosystem properties and community composition. In northeast Iceland several lakes support large-scale midge (Diptera: Chironomidae) emergences that can represent significant N inputs to land when midges die and become incorporated into the soil profile. I hypothesized that the increased N inputs through midge carcass deposition to land would have a bottom-up effect on plant communities, with fast-growing graminoids dominating areas where midge inputs are high and the more typical slow-growing heathland and woody species dominating areas where insect inputs are low. I measured the relationship between midge abundance and plant community composition (structure) and production (function) around seven lakes with varying levels of midge emergence. I measured midge deposition and a series of plant variables (functional group abundance, biomass, root production, and leaf tissue N) at 5, 50, 150, and 300 to 500 m from the lakeshore. As predicted, midge inputs were positively related to changes in graminoid communities, and negatively related to heath and woody communities. These findings indicate that graminoids are able to not only take advantage of midge-derived nutrients, but they do so more than other plant functional groups. Insect movement to land during emergence can significantly alter surrounding terrestrial plant communities through changes to nutrient availability. Insect deposition onto land may affect plant communities surrounding any aquatic source that supports emergent insects.

INTRODUCTION

Near shore terrestrial ecosystems may respond to the deposition of aquatic-derived nutrients through the emergence and subsequent death of adult aquatic insects onto land (Sanzone et al. 2003, Paetzold et al. 2011, Dreyer et al. 2012). Aquatic insects have had measurable effects on communities surrounding aquatic shorelines (Gratton et al. 2008, Brehme et al. 2009), especially during emergence events. During periods of insect emergence, adult insects move over land to mate and congregate in areas near the shoreline where they alter arthropod food webs as a function of distance from shore (Gratton et al. 2008, Gratton and Zanden 2009). Near the lakeshore, large quantities of emergent insects can alter not only the abundance of arthropods (Dreyer et al. 2012), but also the feeding habits of riparian insect predators (Sanzone et al. 2003, Akamatsu and Toda 2011) and the consumption of emergent insect carcasses by detritivores (Hoekman et al. 2012). Yet, due to the often synchronized nature of insect emergences and predator satiation responses, predators do not consume a large fraction of emergent aquatic insects. Rather, emergent insects primarily die on land and become part of the decomposer food web and are incorporated into the soil profile. As a consequence, aquatic insects may have the capacity to affect plant communities that respond to changes in soil nutrient availability.

Large populations of insects directly and indirectly affect plant communities. In addition to direct herbivory on plants, the feeding activity of herbivorous insects can result in the deposition of frass (feces) into the soil which can increase rates of decomposition, soil nutrient cycling, and influence the growth of microbial biomass (Lovett and Ruesink 1995, Frost and Hunter 2004, Hillstrom et al. 2010, Kagata and Ohgushi 2012). In addition, insect carcasses falling to the soil may also have bottom-up effects on plant community

composition and nutrient dynamics (Bartons et al. 2012). Massive insect populations seen during extraordinary periodical cicada emergences can alter soil N and plant and detritivore communities (Yang 2004, 2006). Cicada carcasses significantly increased soil nitrate, ammonium, and microbial biomass, consequently altering soil communities and nutrient availability for plant uptake (Yang 2004). Plant communities in areas receiving insect carcass inputs may therefore have access to increased N availability in the soil. Similar to massive insect deposition seen with recurring insect irruptions, insect emergences from bodies of water may similarly increase soil N and have bottom-up effects on plant communities surrounding bodies of water. However, less is known on the effect of non-deliberate biotic N inputs, such as aquatic insect carcasses, on plant communities during large emergence periods.

Aquatic insect emergence from lakes in northeast Iceland can be so high that they obscure visibility with dense mating columns over land, a phenomenon especially apparent at Lake Mývatn. Up to 550 tons of midge biomass (ash-free dry weight) can leave this lake during peak emergence much of which is deposited on land (Lindegaard and Jonasson 1979). As midge carcasses are ~10% N, midge carcass deposition near shore could reach an annual N input rate of up to 0.4-10 kg N ha^{-1} yr^{-1} (Dreyer et.al. *in prep*). This large quantity of N in the form of carcasses to land may drive changes in plant community composition where insect deposition is high.

The plant communities in subarctic northeast Iceland are characterized as heathlands. Heathland communities are composed of dwarf shrubs, low laying heath plant species, graminoids, forbs, and lichens. The primary productivity of subarctic heathlands is low due to limited nutrient availability (Shaver et al. 2001, Wookey et al. 2009, Lavoie et al. 2011,

Gough et al. 2012) and harsh abiotic conditions such as low mean annual temperature, soil moisture and short seasonality (Hobbie and Chapin 1998, Britton et al. 2003, Weintraub and Schimel 2005). Variation in traits, such as woody stem production (woody shrubs) or high leaf turnover (grasses), may affect the extent and speed by which these can plants react to, and compete for, available N (Bret-harte et al. 2001, 2008, Shaver et al. 2001). N from insect carcass deposition during midge emergence periods could be an especially important nutrient source for heathland plant communities, which are often N limited (Mack et al. 2004, Bret-harte et al. 2008).

We hypothesize that aquatic insect inputs will alter the structure and function of the surrounding terrestrial plant community. Due to the large midge emergence around some lakes in northeast Iceland, we expected to find N enrichment and high grass dominance where midge input was high. We expected plant functional groups to respond differently to inputs, as adaptations to low nutrient environments by dwarf shrubs and low-laying heathland plants make them poor competitors in nutrient rich areas (Jägerbrand et al. 2009). In order to test this hypothesis, we investigated how plant community assemblages, aboveground biomass, leaf nutrient concentrations, and belowground production varied in relation to midge abundance at seven lakes in northeast Iceland. Midge abundance varied across a gradient of "low" midge (oligotrophic) lakes to "high" midge (eutrophic) lakes and as a function of proximity to shore, with more midges near shore. We predicted a higher relative abundance of fast growing herbaceous plants and increased above and belowground productivity in locations that received high midge deposition.

METHODS

Study System

This study was conducted in northeastern Iceland, an area with low mean annual temperature (2°C) and low mean annual precipitation (~400 mm) (Einarsson 1979). The high latitude (65.6°N) results in a short growing season, spanning from late May to late August. These abiotic factors support heathland plant communities consisting of dwarf shrubs and graminoid species that are generally well adapted to harsh environments with low productivity and nutrient limitation (Chapin and Shaver 1988, Vuuren et al. 1992, Aerts 1999, Lavoie et al. 2011). Common plant species were grouped into four functional groups based on their physiology: heath, woody, graminoids and forbs (Table 1). Heath plants were classified as low-laying, prostrate species and included such plant species as *Vaccinium uliginosum* and *Arctostaphylos uva-usi*. Woody plants (sometimes termed "shrub land") included large, upright shrubs such as *Betula nana* and *Salix phylicifolia.* Graminoids included grasses and sedges while forbs were classified as herbaceous flowering plants. Lichens and mosses were present but not included in analyses.

Estimating midge inputs to land

To measure midge deposition to land, we set up midge infall traps at the seven lakes in August 2009 along transects perpendicular to the shoreline. At most lakes we delineated two transects, but at the largest lake, Mývatn, we established eleven transects to account for the greater spatial variability around the lake. Transects had infall sampling stations at 5, 50, 150, and 300 to 500 m from shore. Infall traps consisted of plastic cups (1000 ml; 11 cm diameter) filled 1/3 full with a 1:1 mixture of ethylene glycol and water and a drop of

unscented detergent. Traps were affixed to 1-m tall stakes at each distance station along transects and were deployed continuously from late May to early August. Midges were removed and counted about every 10 days. Total counts of individual midges were measured at each station and converted to midge biomass per cup (average midge 0.001 mg dry weight) to determine total midge deposition rates per year.

Measuring plant community response to midge inputs

To understand how midge deposition influences plant community structure, we measured plant species cover and aboveground biomass across the midge deposition gradient. To measure plant cover at each sampling station along the transect, 20 1-m^2 quadrats were arrayed in a grid within 30 x 30 m area near the midge infall trap. In each m^2 quadrat, we identified the dominant plant functional group as the group covering more than 50% of the quadrat. If the functional group was identified as "heath" or "woody", we recorded the dominant plant to species. Relative cover for each of the four major plant functional groups (woody, heath, graminoid, forb) was calculated as a proportion of 20 total measurements at each transect station.

Aboveground plant biomass was measured indirectly through measurements of leaf area index (LAI) within the same 1-m^2 quadrat used for plant cover estimates. LAI was calculated from eight total measurements of intercepted photosynthetically active radiation (iPAR) taken above and below the plant canopy, according to Campbell and Norman (1998) using an AccuPAR 80 ceptometer (Decagon Devices, Seattle, WA). To estimate aboveground biomass using LAI, we had to first correlate iPAR to site measurements of aboveground plant biomass. We measured iPAR in 1-m^2 plots at 30 sites away from our main

sampling transect, where different functional groups were dominant. After iPAR was measured, we harvested all aboveground biomass within the quadrat. Biomass was sorted into wood, dead, and green biomass, then dried to constant mass at 60° C and weighed. We then calculated the amount of green biomass per m^2 and correlated it to measurements of LAI. The best relationship between LAI and dried green biomass was a power function, which we used to predict green biomass as: Biomass = $348.56 \times LAI^{0.73}$; $R^2=0.71$. However, three abundant heath species, (*Empetrum nigrum, Vaccinium uliginosum,* and *Arctostaphylos uva-ursi*), were lying so low to the ground that the ceptometer could not be placed completely beneath the green canopy and LAI could not be accurately measured. For these particular species we harvested, dried, and weighed biomass from six separate quadrats. Because variability among quadrats was low, we were able to generate an average value of biomass for these functional groups and apply it to all samples where heath species were found to be dominant. Average green biomass for each of the four functional groups was then calculated on a square meter basis. Aboveground biomass was calculated as the average of 20 total biomass estimates multiplied by the percent cover for a given functional group.

Determining plant tissue nutrient content

To measure the N and C content in the aboveground biomass tissues, green leaf biomass was separated by species, oven dried, and ground to a fine powder. Ground samples were rolled in 5×9-mm tin capsules and analyzed for C and N using a Flash EA1112 elemental analyzer (ThermoQuest Italia, Rodano, Italy). C and N were averaged at each station for species that dominated a majority of the biomass. Dominant species included *Betula nana* (dwarf birch, woody)*, Vaccinium uliginosum* (heath), and grasses (graminoids).

Forb species composition was highly variable among plots so tissue C and N for these species was removed in our analysis to avoid conflating species differences with midge deposition effects.

Measuring root production rates in relation to midge inputs

To calculate relationships between midge deposition and belowground net primary productivity (BNPP), we measured BNPP using ingrowth cores (Fahey et.al. 1999), in which a porous mesh core was filled with clean native soil and left in the ground for roots to penetrate. Beginning in the spring of 2008, two 5×15-cm soil cores were taken at each transect station. Soil cores were returned to the lab and sieved to 2-mm to remove root material and rocks. Plastic ingrowth root core sleeves were filled with the clean sieved soil and re-inserted into the ground near where native soil was collected. A few cores from areas inaccessible at 5-m due to rough or impassible terrain were inserted 20-m away from the lakeshore and treated as 5-m in later calculations. Because of the short growing season, a subset of cores was harvested after one growing season while others remained in the ground for two seasons.

During removal and final harvest of these cores, care was taken to cut away the soil and roots outside of the plastic to minimize loss of roots from inside the core. Mesh cores were then cut open and the contents sieved to 2-mm to remove loose soil. Roots were carefully washed using a 0.83-mm sieve to reduce fine root loss, dried at a constant temperature of 62 °C, and weighed on a Shimadzu AY120 microbalance to the nearest 0.1 mg. We calculated BNPP as the total root biomass produced per year, accounting for a yearly fine root turnover of 64% (Aerts et al. 1992). Cores were averaged when multiple cores were recovered at a single station.

Data analysis

Relationships between midge deposition and plant response were analyzed using linear mixed-effects models (LME, RStudio 0.98.490). Plant cover, aboveground biomass, belowground biomass, and leaf C:N were analyzed separately as a function of average annual midge deposition. All models included "site" and "transect" as random effects. To conform to model assumptions, cover data were converted to proportions and arcsine-square root transformed (Crawley 2002). Aboveground biomass, BNPP and midge infall data were log transformed. Significance of fixed factors was determined at the $P<0.05$ level.

RESULTS

Along the wide gradient of midge deposition observed across the lakes we examined in northeastern Iceland, there was a consistent positive relationship between midge deposition and the abundance and biomass of grasses, often at the expense of heath and woody species (Table 2A and B, Fig. 1 and 2). Relationships were particularly strong at Lake Mývatn, where midge deposition was higher compared to all other lakes (Fig. 1 and 2). While there was a substantial displacement of heath and woody cover by graminoids in high midge areas (Fig.1), the biomass produced for each functional group followed slightly different trends. Graminoid biomass increased where midge deposition was high (Fig. 2D). Woody plants produced significantly more biomass when midge deposition was high, thus providing fewer but larger woody shrubs as midges became more abundant (Table 2B, Fig. 2B). Heath biomass remained invariant relative to midge deposition (Table 2B). However, due to the high mass and density of leaves per area, heath biomass was consistently highest across all functional groups. There was also a significant positive relationship between

midge deposition and total belowground net primary production (Fig.3; Table 2C). BNPP was generally higher in sites surrounding Lake Mývatn (Fig.3).

Where midge deposition was high, we also observed measurable increases in plant leaf quality (lower C:N) for both grasses and *V. uliginosum* (Table 2D, Fig. 4A and B). In general, the woody shrub *B. nana* had lower overall leaf C:N compared to grass and *V. uliginosum* although woody C:N was not associated with changes in midge deposition (Fig. 4C). Where midge abundance was high, heath and graminoid plant species had increased leaf N concentrations.

DISCUSSION

Despite the fact that emergent aquatic insects can represent a significant input of C and N to land, few studies have quantitatively measured how plant communities vary as a function of insect deposition. Around lakes in northeastern Iceland, there is an overall positive association between the relative abundance of aquatic insects and subsequent changes in plant communities and ecosystem properties. In areas where midge deposition was low, either at oligotrophic lakes or at distances far away from shore (≥ 200 m), there was a greater dominance of slow growing shrubs and vegetation typical of subarctic heathlands. Examples of common plant species include the low growing woody shrubs such as *Betula nana*, *Salix phylicifolia* and *Salix lanata*, and the heath plant species such as *Empetrum nigrum*, *Vaccinium uliginosum* and *Arctostaphylos uva-usi*. Grasses and forbs dominated areas where midge abundance was high, either near productive eutrophic lakes (such as Mývatn) or near areas closer to lakeshore.

Though we show the existence of midge-plant relationships, the correlative nature of this study limits our ability to assess direct midge carcass effects on plant communities and may interact with other alterations on the landscape. Since many of the areas surrounding our sites are also open to sheep grazing, disturbance by sheep and a history of woody biomass removal to improve forage production by farmers may also influence the vegetation patterns along the midge-deposition gradient. However, the overall strong and generally positive midge influence on graminoid biomass, abundance, and leaf N across the landscape indicates that plant communities change relative to midge deposition.

We worked across a natural environmental gradient of aquatic insect deposition to land, taking advantage of the variability in lake productivity in northeastern Iceland. Observed influence on midge-plant relationships were particularly strong at Lake Mývatn, where high midge deposition defined relationships between midge inputs and vegetation patterns otherwise obscured at lakes that did not produce as many midges. As midge deposition at Mývatn could provide up to 10 kg N ha^{-1} yr^{-1} to land within 50 m of shore (Dreyer et.al. *in prep*), additional midge N input could triple the average background atmospheric deposition rates estimated in arctic environments (3-5 kg N ha^{-1}; Sievering et al. 1996). Thus, we suggest that the influx of N associated with insect carcass deposition to land in these areas was sufficient to have a bottom-up effect on plant communities comparable to those observed in atmospheric N deposition or N fertilization experiments.

Measured changes in plant community composition along the midge deposition-gradient were likely a biological response of these plant communities to the enhanced levels of available N. The relatively rapid tissue turnover of most herbaceous graminoid and forb species positions them to respond quickly to nutrient input compared to other slow-growing

heath and woody species (Aerts and Berendse 1989, Eckstein and Karlsson 1997, Jägerbrand et al. 2009) and likely explains some of the trends we see developing among plant functional groups. We did not observe any increase in abundance of the woody shrub *Betula nana*, even though other studies found *Betula* species to thrive in areas of nutrient addition (Bret-harte et al. 2001, Shaver et al. 2001, Weintraub and Schimel 2005). Because midge emergence has occurred yearly for hundreds of years, we suspect this discrepancy in abundance is due to not only a long-term progression of plant community shifts, but also the overall competitive advantage grasses have over woody shrubs in this area.

The competitive ability of heath and woody species under high nutrient condition is low, placing these species at a disadvantage in nutrient rich areas (Richardson et al. 2002). When nutrient conditions are high, graminoids tend to have competitive advantage over woody and heath plant species. We observe this not only in enhanced grass biomass and abundance, but also in increased grass tissue quality when midge deposition is high. Even so, this does not mean woody and heath plants cannot benefit from nutrient inputs. Our results partially support previous research showing that *Betula* can benefit from high levels of nutrient addition, but only through increases in biomass, not relative cover. Allocation to growth (biomass) versus competitive ability (abundance) when nutrient availability is high may be driving the positive trends we observe in woody biomass. Woody plant characteristics such as low leaf production costs, high leaf turnover, and greater nutrient allocation to woody stem production (Shaver et al. 2001), may be advantageous when nutrients become available. This nutrient allocation to biomass production, verses propagation, may explain the increased standing biomass of woody plant species in areas of high midge inputs. Conversely, where midge deposition increased, heath plant species put

substantial energy into enhancing plant tissue quality and not into increasing biomass production or abundance. Thus, the varying physiological response of subarctic heath and woody species to midge carcass deposition is likely due to different species adaptations to reduce nutrient loss (Aerts 1999).

When we remove variation among plant functional groups, we found midge-associated changes in plant root production. We suspect the increased BNPP rates observed in our study is likely due to the increased dominance of graminoids. Biologically, graminoids are known for fine root production, and when the number of graminoid species are high, fine root production tends to be high (Sullivan et al. 2007). This relationship can escalate when external nutrients are added into a system via fertilization, due to the strong adaptive response of graminoids to nutrient availability (Nadelhoffer et al. 2002). While plant response to carcass inputs can occur after a single year of carcass addition in temperate systems (Yang 2004, 2006), we show that long-term midge deposition may have continued strong bottom-up effects on plant community function in these northern lake landscapes.

In summary, aquatic insect carcasses can modify terrestrial plant community composition and ecosystem function. Though Lake Mývatn was an important contributor emphasizing plant-midge relationships, we still observed significant relationships linking aquatic midge deposition and terrestrial plant communities among various levels of lake productivity. Our study illustrates that near shore plant communities can be modified by aquatic insect carcass deposition. Plant communities near any lake or stream that fosters emergent insect populations may be similarly affected by the emergence and death of these insects on land.

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LITERATURE CITED:

Aerts R, Bakker C, De Caluwe H. 1992. Root turnover as determinant of the cycling of C , N , and P in a dry heathland ecosystem. Biogeochemistry 15:175–90.

Aerts R. 1999. Interspecific competition in natural plant communities : mechanisms, trade-offs and plant–soil feedbacks. J Exp Bot 50:29–37.

Akamatsu F, Toda H. 2011. Aquatic subsidies transport anthropogenic nitrogen to riparian spiders. Environ Pollut 159:1390–7

Barton PS, Cunningham S a, Lindenmayer DB, Manning AD. 2012. The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. Oecologia 171, 761-772.

Brehme CS, Boarman WI, Hathaway S a., Herring A, Lyren L, Mendelsohn M, Pease K, Rahn M, Rochester C, Stokes D, Turschak G, Fisher RN. 2009. Spatial and temporal patterns across an ecological boundary: Allochthonous effects of a young saltwater lake on a desert ecosystem. J Arid Environ 73:811–20.

Bret-harte MS, Mack MC, Goldsmith GR, Sloan DB, Demarco J, Shaver GR, Ray PM, Biesinger Z, Chapin FS. 2008. Plant functional types do not predict biomass responses to removal and fertilization in Alaskan tussock tundra. J Ecol 96:713–26.

Bret-harte MS, Shaver GR, Zoerner JP, Johnstone JF, Wagner JL, Chavez AS, Gunkelman RF, Lippert S, Laundre J. 2001. Developmental plasticity allows betula nana to dominate tundra subjected to an altered environment. Ecology 82:18–32.

Britton A, Marrs R, Pakeman R, Carey P. 2003. The influence of soil-type , drought and nitrogen addition on interactions between Calluna vulgaris and Deschampsia flexuosa : implications for heathland regeneration. Plant Ecol 166:93–105.

Campell, G., John Norman (1998). *An Introduction to Environmental Biophysics*. Springer Science + Business Media.inc. New York, NY. USA

Chapin FS, Shaver GR. 1988. Differences in carbon and nutrient fractions among arctic growth forms. Oecologia 77:506–14.

Dreyer J, Hoekman D, Gratton C. 2012. Lake-derived midges increase abundance of shoreline terrestrial arthropods via multiple trophic pathways. Oikos 121:252–8.

Einarsson M. 1979. Climatic conditions of the Lake Mývatn area. Oikos 32:29–37.

Fahey TJ, Bledsoe CS, Day FP, Ruess RW, Smucker AM (1999) Fine root production and demography. In: Robertson GP, Bledsoe CS, Coleman D, Sollins P (eds) Standard soil methods for long-term ecological research. Oxford University Press, New York, pp 437-455

Frost C, Hunter M. 2004. Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. Ecology 85:3335–47.

Gough L, Moore J, Shaver G. 2012. Above-and belowground responses of arctic tundra ecosystems to altered soil nutrients and mammalian herbivory. Ecology 93:1683–94.

Gratton C, Donaldson J, Vander Zanden MJ. 2008. Ecosystem Linkages Between Lakes and the Surrounding Terrestrial Landscape in Northeast Iceland. Ecosystems 11:764–74.

Gratton C, Zanden M. 2009. Flux of aquatic insect productivity to land: comparison of lentic and lotic ecosystems. Ecology 90:2689–99.

Hillstrom M, Meehan TD, Kelly K, Lindroth RL. 2010. Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO2 and O3. Plant Soil 336:75–85.

Hobbie S, Chapin FS. 1998. The response of tundra plant biomass, aboveground production, nitrogen, and CO2 flux to experimental warming. Ecology 79:1526–44.

Hoekman D, Bartrons M, Gratton C. 2012. Ecosystem linkages revealed by experimental lake-derived isotope signal in heathland food webs. Oecologia 170:735–43.

Jägerbrand AK, Alatalo JM, Chrimes D, Molau U. 2009. Plant community responses to 5 years of simulated climate change in meadow and heath ecosystems at a subarctic-alpine site. Oecologia 161:601–10.

Kagata H, Ohgushi T. 2012. Non-additive effects of leaf litter and insect frass mixture on decomposition processes. Ecol Res 27:69–75.

Lavoie M, Mack MC, Schuur E a. G. 2011. Effects of elevated nitrogen and temperature on carbon and nitrogen dynamics in Alaskan arctic and boreal soils. J Geophys Res 116:1–14.

Lindegaard C, Jonasson PM. 1979. Abundance, population dynamics and production of zoobenthos in Lake Mývatn, Iceland. Oikos 32:202–27.

Lovett G, Ruesink A. 1995. Carbon and nitrogen mineralization from decomposing gypsy moth frass. Oecologia 104:133–8.

Mack MC, Schuur EAG, Bret-harte MS. 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 431:658–61.

Nadelhoffer K, Johnson L, Laundre J, Giblin AE, Shaver GR. 2002. Fine root production and nutrient content in wet and moist arctic tundras as influenced by chronic fertilization. Plant Soil 242:107–13.

Paetzold A, Smith M, Warren PH, Maltby L. 2011. Environmental impact propagated by cross-system subsidy: Chronic stream pollution controls riparian spider populations. Ecology 92:1711–6.

Richardson SJ, Press MC, Parsons AN, Hartley SE. 2002. How do nutrients and warming impact on plant communities and their insect herbivores? A 9-year study from a sub-Arctic heath. J Ecol 90:544–56.

Sanzone DM, Meyer JL, Marti E, Gardiner EP, Tank JL, Grimm NB. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. Oecologia 134:238–50.

Shaver GR, Bret-harte MS, Jones MH, Johnstone J, Gough L, Laundre J, Chapin FS. 2001. Species composition interacts with fertilizer to control long-term change in tundra productivity. Ecology 82:3163–81.

Sievering H, Rusch D, Marquez L. 1996. Nitric acid, particulate nitrate and ammonium in the continental free troposphere: Nitrogen deposition to an alpine tundra ecosystem. Atmos Environ 30:2527–37.

Sullivan PF, Sommerkorn M, Rueth HM, Nadelhoffer KJ, Shaver GR, Welker JM. 2007. Climate and species affect fine root production with long-term fertilization in acidic tussock tundra near Toolik Lake, Alaska. Oecologia 153:643–52.

Vuuren M Van, Aerts E, Berendse F, Visser W De. 1992. Nitrogen mineralization in heathland ecosystems dominated by different plant species. Biogeochemistry 16:151–66.

Weintraub MN, Schimel JP. 2005. Nitrogen Cycling and the Spread of Shrubs Control Changes in the Carbon Balance of Arctic Tundra Ecosystems. Bioscience 55.

Wookey P a., Aerts R, Bardgett RD, Baptist F, Bråthen KA, Cornelissen JHC, Gough L, Hartley IP, Hopkins DW, Lavorel S, Shaver GR. 2009. Ecosystem feedbacks and cascade processes: understanding their role in the responses of Arctic and alpine ecosystems to environmental change. Glob Chang Biol 15:1153–72.

Yang L. 2004. Periodical Cicadas as Resource Pulses in North American Forests. Science (80-) 306:1565–7.

Yang LH. 2006. Interactions between a detrital resource pulse and a detritivore community. Oecologia 147:522–32.

TABLES

Table 1. Percentage of dominant Heathland life form (functional group) in one square-meter plots. Functional groups were broken down by species to highlight major species. Measurements were taken along distance transects at 7 lake sites of varying productivity in Northeast Iceland.

Life form	Species/group	Mean % cover
Woody	*Betula nana*	25.64
	Salix phylicifolia	7.69
	Salix lanata	4.66
	Juniperus communis	6.25
	Betula pubescens	1.10
Heath	*Empetrum nigrum*	8.84
	Vaccinium uliginosum	7.60
	Arctostaphylos uva-ursi	5.38
	Calluna vulgaris	4.59
Graminoid	Grasses	30.34
	Sedges and rushes	11.86
	Hayfield grass	6.31
	Roadside grass	4.61
Forb	*Galium verum*	10.27
	Achillea millefolium	4.57
	Potentilla palustris	2.85
	Geum rivale	1.25
	Equisetum arvense	1.59
	Alchemilla vulgaris	1.55
	Ranunculus acris	1.56
	Dryas octopetala	1.82
	Trifolium repens	0.72
Lichen		3.44
Moss		2.31
Bare	Bare soil or rocks	8.20

Table 2: Coefficients from a liner mixed effects model displaying relationships between log midge infall (g dw/trap/yr) and the following plant community variables taken along distance transects at 7 lake sites of varying productivity in Northeast Iceland: A) proportion plant cover, B) aboveground biomass (g/m^2), C) belowground production, and D) plant tissue C:N. LME model included a random effect of site and transect.

Plant Variable	Functional group	df	t-value	β ± s.e.	
A. Plant Cover[B]	Graminoid	34	19.01	0.07 ±0.01	***
	Woody	31	-1.89	-0.02 ±0.01	†
	Heath	26	-2.01	-0.02 ±0.01	†
	Forb	14	0.38	0.01 ±0.02	*NS*
B. Aboveground Biomass[A]	Graminoid	34	4.09	0.07 ±0.02	***
	Woody	31	3.63	0.04 ±0.01	***
	Heath	26	-0.90	-0.01 ±0.01	*NS*
	Forb	13	2.98	0.11 ±0.04	**
C. root BNPP[A]	all	21	5.05	0.59 ±0.12	***
D. % leaf tissue C:N	Graminoid (*grass)*	22	-3.91	-1.46 ±0.37	***
	Woody *(Betula nana)*	6	-1.87	-0.41 ±0.22	NS
	Heath *(Vaccinium uliginosum)*	8	-2.32	-0.76 ±0.33	*

[A] log transformation

[B] arcsin√cover x 0.01

NS=not significant, †p<0.1, *p<0.05, **p<0.01, ***p<0.001

FIGURES:

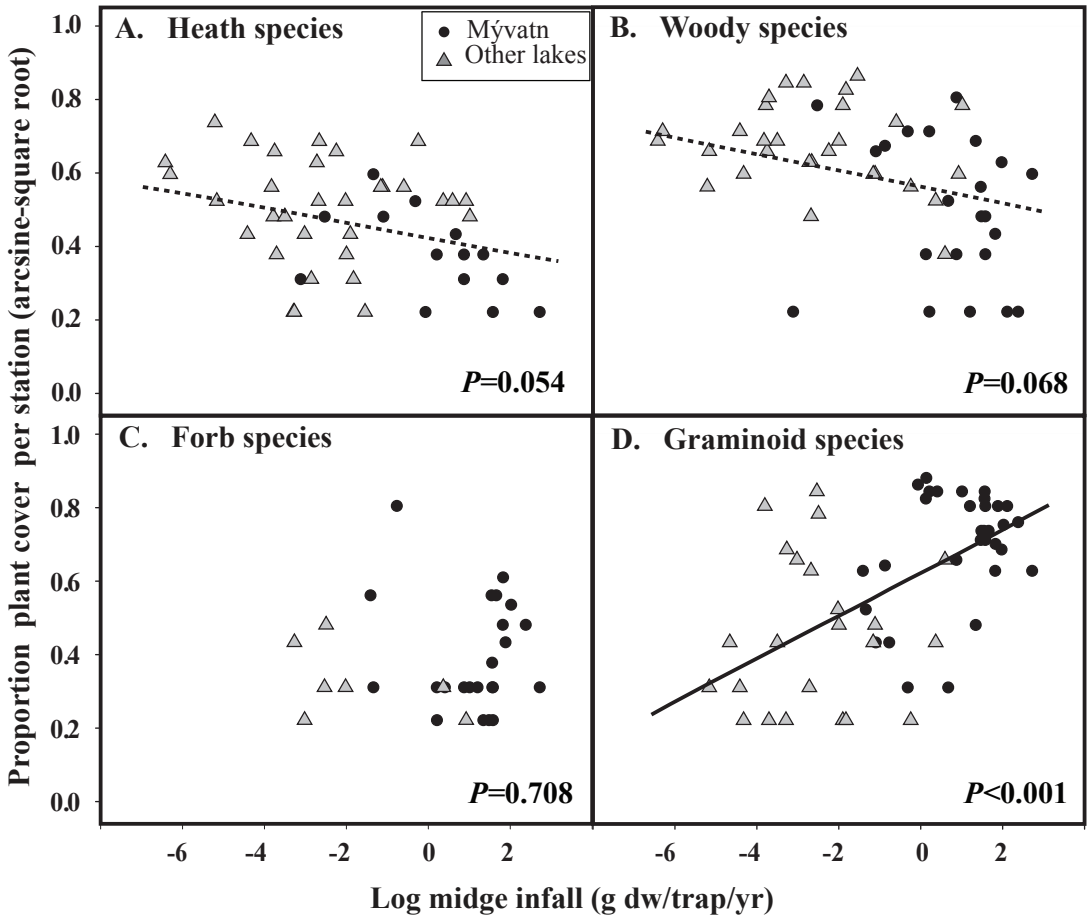

Figure 1: Relationships between midge infall and proportion plant cover sampled along distance transects (0-500m) at 7 lakes of varying productivity in Northeast Iceland, organized by the following functional groups: A) Heath, B) Woody, C) Forb, D) Graminoid. Symbols separate out the highly productive lake Mývatn from the other lakes. Soild lines represent a significant relationship at $p<0.05$ and dotted lines represent marginally significant relationships at $p<0.1$. An absence of a line represents an insignificant relationship.

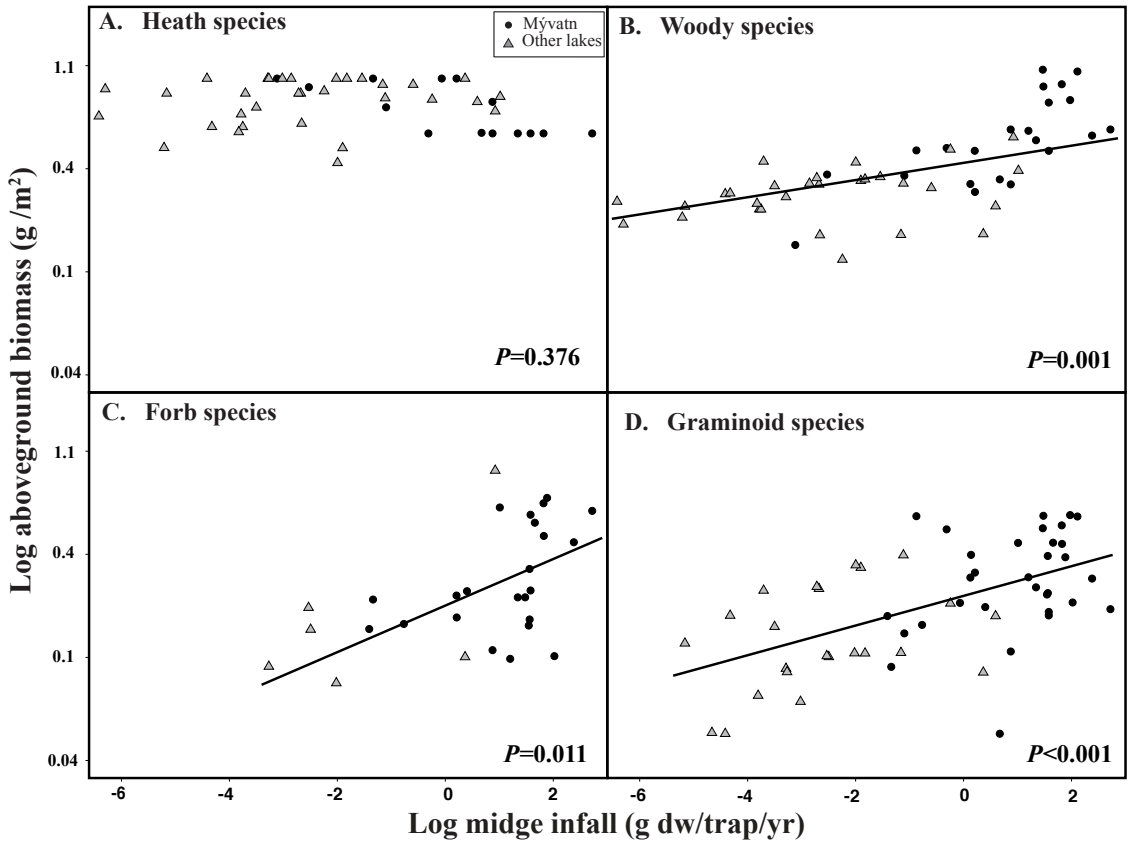

Figure 2: Relationship between midge infall on aboveground biomass sampled along distance transects (0-500m) at 7 lakes of varying productivity in Northeast Iceland, organized by the following functional groups: A) Heath, B) Woody, C) Forb, D) Graminoid. Symbols separate out the highly productive lake Mývatn from the other lakes. Each sampling station along the distance transects covered a 30 x 30-m^2 area. Soild lines represent a significant relationship at $P<0.05$ level. An absence of a line represents an non-significant ($P>0.05$) relationship. Stations at Mývatn are black cicles, while other lakes are gray triangles.

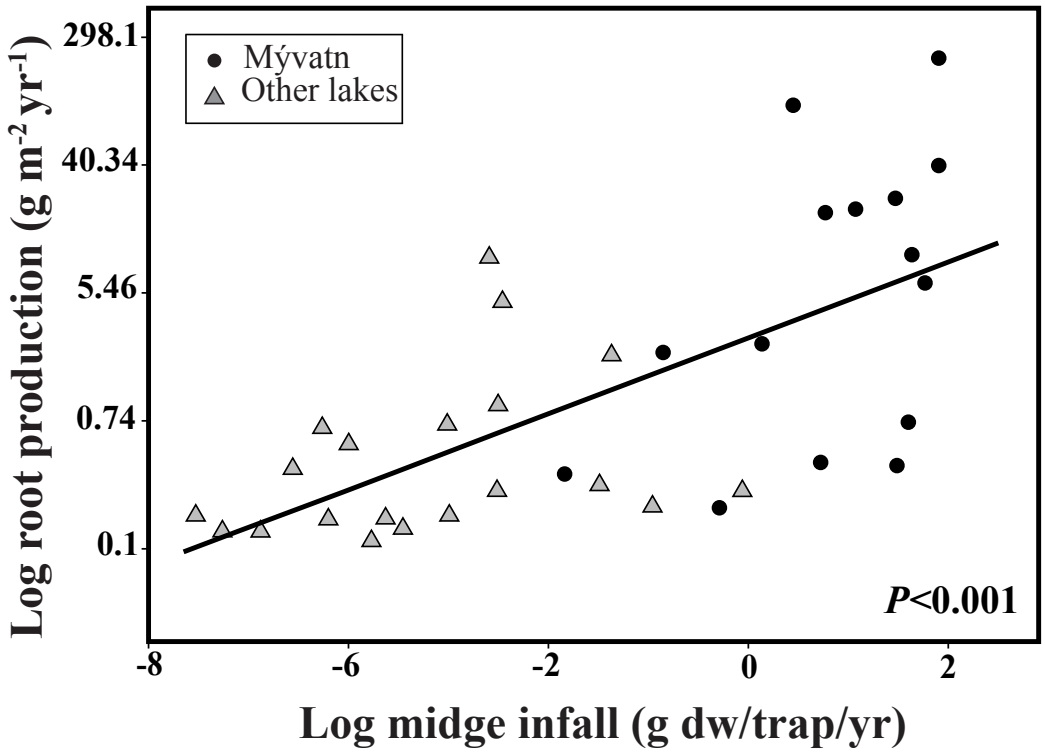

Figure 3: Relationships between midge infall and total belowground net primary production along distance transects (0-500m) at 7 lakes of varying productivity in Northeast Iceland. Symbols separate out the highly productive lake Mývatn from the other lakes. Soild lines represent a significant relationship at $P<0.05$ level.

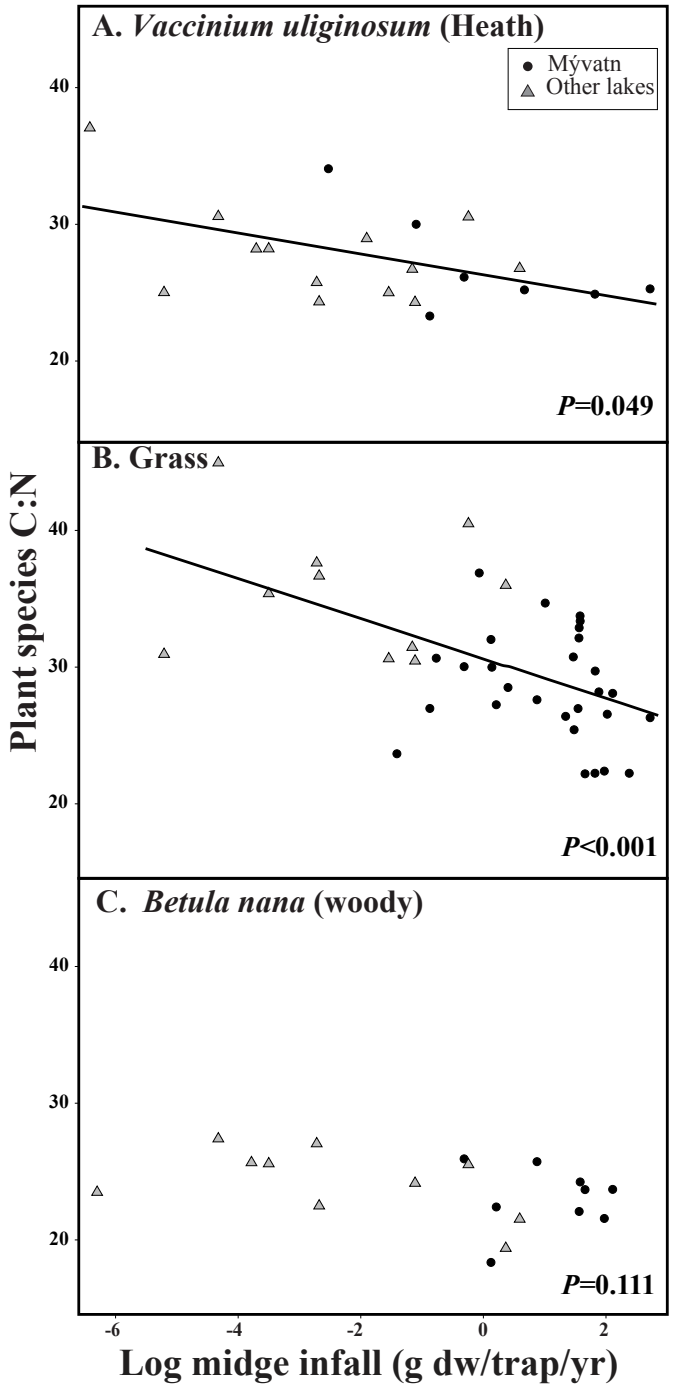

Figure 4: Relationships between midge infall on plant C:N for three dominant plant species taken along distance transects (0-500m) at 7 lakes of varying productivity in Northeast Iceland. Symbols separate out the highly productive lake Mývatn from the other lakes. Soild lines represent a significant relationship at $p<0.05$ level. An absence of a line represents an insignificant relationship.

Chapter 2. Insect carcasses increase soil microbial activity and alter microbial community composition in a heathland ecosystem

ABSTRACT

Movement of aquatic insects from lakes to land can affect terrestrial community interactions. However, as aquatic insects die on land, little is known of how they affect soil communities and ecosystem processes. At Lake Mývatn in northeastern Iceland, aquatic midges represent a high quantity and quality (low C:N) source of organic input to terrestrial soils which we hypothesize will increase decomposition rates and alter microbial community structure. To test the hypothesis, I deposited midge (Chironomidae) carcasses at two different rates (multi-year "pressed" addition and single year "pulsed" addition) on 1-m^2 heathland plots that normally do not experience midge inputs. I collected soils from these plots and measured soil CO_2 flux and ^{13}C-CO_2 during a month long lab incubation of both the mineral soil and organic top-layer sampled from the pressed and pulsed midge addition plots. In addition, I examined microbial community composition in the mineral soil via phospholipid fatty acid extractions (PLFA). Multi-year pressed midge addition significantly increased CO_2 flux over time in the organic top layer, but not in the mineral soil. CO_2 flux in the organic top layer was significantly different among all midge treatments and highest in the pressed midge addition treatment. Pressed midge addition had significantly higher $\delta^{13}C$ values respired across soil types, suggesting that multiple years of midge addition altered the C source for microbes. Pressed midge addition also altered microbial community abundance and increased total microbial biomass. However, the ratio of fungi to bacteria was not affected by midge addition, suggesting that both bacteria and fungi benefited from midge addition. I conclude that yearly additions of insect carcasses are an important source of organic inputs to the soil

and positively affect the structure and function of microbial communities in heathland ecosystems. Where insect carcasses are deposited to terrestrial ecosystems through aquatic insect emergence, we can expect modifications in surrounding soil community composition and decomposition rates.

INTRODUCTION

Nutrient transfer from aquatic to terrestrial environments can be driven by organisms that move across ecosystem boundaries (Lundberg and Moberg 2003, Nowlin et al. 2008). Biotic movement onto land can alter terrestrial ecosystem productivity by either providing a direct food source to land predators (Akamatsu and Toda 2011, Hoekman et al. 2012) or indirectly through soil enrichment and subsequent plant enrichment (Anderson and Polis 1999, Brehme et al. 2009). Similar to the movement of aquatic nutrients to land by larger organisms such as seabirds (Anderson and Polis 1999), smaller organisms like insects can transfer a substantial amount of nutrients from aquatic to terrestrial ecosystems. Insect movement across such boundaries is an important ecological resource pathway for terrestrial communities (Malison and Baxter 2010, Paetzold et al. 2011, Akamatsu and Toda 2011, Dreyer et al. 2012), moving large amounts of nutrients, as insect biomass, to adjacent ecosystems (Gratton et al. 2008, Gratton and Zanden 2009, Rundio and Lindley 2012). Near-shore communities can respond to the increased insect deposition in a number of ways, including shifts in arthropod food-web structure (Jonsson and Wardle 2009, Paetzold et al. 2011, Akamatsu and Toda 2011) and shifts in plant communities where insect carcass inputs are high (Raudenbush et.al. *in prep*). Despite evidence for aboveground terrestrial responses to insect movement, the role of insect deposition on belowground community structure and function is poorly understood, even though large populations of insects can move overland and substantially alter soil communities.

Massive insect swarms can affect soil nutrient concentrations and soil microbial community structure. For example, during herbivore outbreaks, insect feces (frass) fall to the ground and increase soil N and carbon (C) pools (Frost and Hunter 2004), decomposition

rates (Kagata and Ohgushi 2012), and soil respiration (Hillstrom et al. 2010). Similar to frass inputs, cicadae carcass deposition to the soil can also change soil microbial communities after a single pulse of carcasses, as measured by increased available N and soil community biomass (Yang 2004). The effects of insect carcass deposition on soil communities may be especially apparent during large aquatic insect emergence periods, as aquatic insect deposition can move large quantities of organic N and C to land. In Wisconsin, USA, it has been modeled that 6,800 metric tons of C emerge from aquatic sources, with a range of 0.007-52g C m^{-2} yr^{-1} leaving lakes and depositing onto land (Bartrons et al. 2013). As aquatic insect emergence provides both high quality and high quantity carcasses to land, we expect insect carcasses to change soil communities.

Soil microbial communities can respond quickly to high quantity and high quality organic C and N inputs (Kallenbach and Grandy 2011), though the response of specific microbial groups may differ. Biologically, fungi and bacteria have different functional roles in soil ecosystems, and can react differently to nutrient addition (Strickland and Rousk 2010). Since many ecosystems are N limited, N addition can place limitations on C availability. Easily accessible, or labile, C can be used by both bacteria and fungi, while more complex substrates that require additional energy to break down tend to benefit fungal communities (Moore et al. 2004).

As fungi have developed hyphae networks that can effectively uptake both recalcitrant and plant-derived C depending on accessibility and conditions (Vanderwal et al. 2006, Tavi et al. 2013), fungi can thrive under poor nutrient conditions (Nilsson et al. 2011). However, because fungi have a higher nutrient demand and can receive nutrients indirectly through plant-associated symbiosis, bacteria tend to respond quickly and dominate soil

communities receiving labile nutrient additions (Bardgett et al. 1995, Lennon and Cottingham 2008, Krumins et al. 2009). Studies measuring the effects of organic N and C inputs on microbial communities have focused primarily on the availability of plant-derived or fertilization inputs (Demoling et al. 2008, Lavoie et al. 2011, Stark et al. 2011), even though the importance of natural insect carcass deposition has been identified (Yang 2004). We argue that aquatic insect carcasses represent an important natural source of C and N to terrestrial systems and that insect carcass deposition will alter soil microbial community composition and shift communities towards bacterial dominance.

Microbial communities drive soil nutrient turnover, facilitating the decomposition of soil organic matter (SOM) and the turnover of soil organic carbon (SOC). As microbes break down SOM, they respire CO_2, providing a measurable indicator of microbial decomposition rate. Decomposition rates can be affected by the quality and quantity of organic nutrient inputs available to microbes (Nowinski et al. 2008, Cusack et al. 2011, Esch et al. 2012). Insect carcasses can be a rich nutrient source (6-12% N; Fagan et al. 2002) for microbes, and may provide available nutrients to otherwise nutrient limited soils. These additions may have substantial effects on the ability for the microbial community to process nutrients. We expect that nutrient addition in the form of midge carcasses will reduce nutrient limitation for soil microbes and stimulate greater rates of decomposition. Due to the labile nature of midge carcasses, we expect that microbial communities are directly consuming midge carcasses during decomposition. As midges have a distinct $\delta^{13}C$ from that of soil and plant, we expect that the $\delta^{13}C$ in respired CO_2 to resemble midge ^{13}C values in plots that receive a midge treatment.

The extent of soil microbial community responses to carcass deposition could fluctuate based on the periodicity of aquatic insect emergence. Inconsistent carcass deposition rates onto land affect the timing of resource availability, which may change soil community responses to nutrient availability (Schwinning and Sala 2004). A resource that arrives in single discrete pulses, like cicada carcasses, can have different effects on ecosystem function and structure than a press (consistent or reoccurring) resource input (Leroux and Loreau 2011). Pulsed resources can have major short-term effects on invertebrate abundance and species diversity (Yee and Juliano 2012) that may last into future years (Gratton and Denno 2003), while pressed resource inputs provide a continuous source of nutrients and can have longer-lasting effects on communities (Hoekman et al. 2012). Due to the strong effect of annual midge carcass availability on terrestrial arthropod abundance and diets (Hoekman et al. 2011, 2012), we expected soil community structure and ecosystem rates to change substantially with a pressed resource input and to temporarily change the year a pulsed resource was added.

We measured the direct effects of insect carcass addition to a terrestrial ecosystem in northeastern Iceland in order to address the question of how aquatic insect carcasses affect terrestrial soil microbial activity and community composition. Dense clouds of midges (Chironomidae) cover the land during yearly midge emergence from Lake Mývatn ("midge lake") which is particularly famous for massive midge emergence events, depositing an estimated 0.4-10 kg N ha^{-1} yr^{-1} to near shore environments (Dreyer et.al. *in prep*) from midge carcasses (~10%N; Gratton et al. 2008). To understand the role of midge carcasses in soil community function and structure, we experimentally added midges to plots in order to mimic the density of carcass deposition from Mývatn at a nearby site with very low natural

midge deposition. Our objectives were to directly measure the effect of midge carcass addition on decomposition rates and soil microbial community composition and biomass. We expected midge carcasses to increase decomposition rates. We also expected carcass addition to promote an increase in total microbial biomass and a shift from fungi to bacteria dominated communities.

METHODS

Midge-addition experiment

To measure the direct effects of midge carcass addition on microbial communities, we performed a midge-addition experiment. Experimental sites were located approximately 5 km from Mývatn, near a low-midge lake in northeast Iceland (Lake Helluvaðstjörn; 65.6°N, 17°W). Helluvaðstjörn was used because it has no recent history (>800 yrs) of high midge populations and therefore soils in these areas represent a low-midge background. Soils in this region are porous, freely drained andosols (Arnalds 2004) with an estimated organic C content of 3.3% (Óskarsson et al. 2004). While the top layer of the soil profile has high organic matter (OM) content chemically similar to that of leaf litter (Rumpel et al. 2011), the andic properties of the soil, such as high allophane content, keep OM from being decomposed quickly (Gudmundsson et al. 2004). The subarctic climatic in northeast Iceland provides a short plant growing season, ranging from late May to early September, and support soils with low rates of nutrient cycling and N limitation (Arnalds and Kimble 2001).

Adjacent to a low-midge lake, lake Helluvaðstjörn, we hand added midge carcasses to 1 m^2 plots (~50 m from shore; details can be found in Hoekman et. al. 2011). Midges were previously collected during peak emergences at Lake Mývatn, brought to the lab and dried to

constant mass at 50 ˚C. The total mass of insect carcasses added to plots (150 g dry weight midges carcasses / m^2) mimicked annual midge deposition in high-input locations at Lake Mývatn. Midges were added in three equally spaced aliquots (1/3 of total added each time) during a 3 week period between late May and mid-June of each year of the study, to coincide with the period of midge emergences in this area. Four treatment plots received a single period of midge carcass input ("pulse") during 2008 (plot n=16), 2009 (n=8), 2010 (n=8) or 2011 (n=8). The other treatment plots received yearly carcass addition for 4 consecutive years ("press", n=16). All treatment plots were paired with a control plot (n=56).

Microbial community composition:

To determine microbial biomass and community structural differences among treatments, we extracted Phospholipid Fatty Acid Analysis (PLFA) from the mineral soil in all midge-addition treatment plots (pulse 2008, 2009, 2010, 2011, press, control) in August 2011. We composited 4 soil cores (2.5x15 cm) taken from each plot (n=48 total) and immediately froze the soil to preserve lipids. We extracted and identified lipids from 3 g of freeze-dried soil, following a modified PLFA extraction method alongside a fatty acid methyl ester analysis (FAME) (Zelles 1999, Balser et al. 2005). Lipids were analyzed on an Agilent 6890 gas chromatograph (Agilent Technologies, Wilmington, DE) and quantified through comparisons of the peak areas (the amount of a given lipid) to two internal standards (9:0 and 19:0; Sigma, St. Louis, MO).

We were interested in measuring midge effects on both the total amount of lipids in each sample (or "total microbial biomass"; μmol/g soil) and the relative community abundance (mol% species/total species). To calculate relative abundance, total microbial biomass values were first converted to mol% for a species (removing any value less than

0.05%) and divided by total species. Final abundance values were arcsine transformed prior to analysis. The specific identification of microbial species was outlined in table 1.

To explore treatment effects on microbial community biomass (μmol/g soil), we ran linear models. We were not only interested in treatment effects on 1) total microbial community biomass, but also in specific treatment effects on 2) fungi biomass, 3) bacterial biomass and 4) the fungi/bacteria ratio. We analyzed treatment effects on these four factors separately. If there was an effect of treatment, we did a multiple comparison using student's t-test. Significance was determined at the $\alpha = 0.05$ level.

Differences in soil microbial community composition between midge addition treatments were visualized using non-metric dimensional scaling (NMDS) and analyzed by PERMANOVA using a Bray-Curtis distance matrix (*vegan* package, RStudio 0.98.490). PERMANOVA models compared relative microbial "species" abundance against treatment. To measure differences among treatment effects, we ran pairwise comparisons and used a Bonferroni correction of $\alpha = 0.0033$. To further quantify treatment effects on the relative abundance of individual microbial species groups (total species abundance, total bacteria, total fungi, gm+,gm-, AMF), we ran ANOVA's to test for treatment effects on the different microbial groups. If treatment was a significant factor, we ran multiple comparisons using student's t-test to determine dissimilarities among treatments. Significance was determined at the $\alpha = 0.05$ level.

Soil incubations

To measure how different midge additions to soil affect initial respired CO_2 and respired CO_2 overtime (i.e., decomposition), we sampled soil from the experimental plots once in early July 2011, 30 days after the last midge carcass addition. We sampled plot-

specific soil from the press and pulse 2011 treatment plots and their paired controls. We used a small (2.5 x15 cm) soil corer to collect four soil cores from each plot. After each core was taken, we immediately removed the top organic layer (typically the top 2-3 cm) and bagged it separately from the remaining mineral soil. We then transported the soil samples to the lab and prepared them for incubations.

We took five site-specific soil cores from just outside the treatment plots and calculated the bulk density and percent moisture at the site. Using bulk density and percent moisture, we standardized the amount of field moisture mineral soil (~80g) that was packed into 160cc cups. Because the organic top layer was variable from plot to plot, we calculated the volume of the organic top layer packed within each individual cup. Cups filled with either the organic layer or the mineral soil (n=96) remained in a darkened lab space at 22 °C as they waited for our lab incubation experiments.

To run in-lab soil incubations, each specimen cup of soil was placed into a gas collection chamber. Chambers were 1 L plastic jars with a screw-top lid outfitted with a rubber septum to allow gas sampling with a syringe. Each chamber (holding the soil cups) was capped and an initial gas sample was taken (T0) with a syringe and injected into an evacuated 5.9 ml glass Exetainter vial (Labco Limited, UK). After 15 minutes, a second gas sample was taken (T15) and placed in a second glass vial. This was repeated again after 30 minutes (T30). After T30, chamber caps were removed and soil cups were moved back into the darkened lab space. Glass vials were processed on a gas chromatograph for measurements of CO_2. From these data we calculated the rate of CO_2 release (total new CO_2 / 30 minutes / g soil material used) on a particular day. Respired CO_2 overtime

(decomposition rate) was estimated over a 29-day period on day 0, 2, 5, 10,17, and 29. This was done separately for mineral soil and organic layer profiles from each experimental plot.

To analyze treatment effects on soil CO_2 respiration (μg C g^{-1} material d^{-1}), we ran a linear mixed effects model (LME). We modeled the change in $\log_e$- CO_2 respiration over time as an ANCOVA with treatment (press, pulse, or control), material (organic or mineral soil), and day (0-29, continuous) as well as treatment x day, material x day and treatment x material interactions and a three-way interaction. The slope parameters represent decay rates (*k*) of CO_2 respiration over the course of the experiment for each of the midge addition treatments and soil types. Individual plots were used as a random effect to account for repeated measurements over time. We used the maximum likelihood (ML) method and fitted models using backwards model selection using the AIC criterion. Our final model lacked a three-way interaction and a day x material interaction.

Stable Isotopes:

In addition to measurements of CO_2 respiration, we also measured the type of C respired by the microbial community. On the first incubation day we took an additional gas sample for C stable isotope analysis. These gas samples were used for C isotope analysis using a Thermo mass spectrometer. Long sample storage (12 months) prior to analysis may have altered absolute $\delta^{13}C$ values. However, all samples were stored under similar conditions making it possible to compare relative treatment differences. We used an analysis of variance (ANOVA) with $\delta^{13}C$ modeled as a function of treatment and soil material. Since we were also interested in determining possible differences among treatments and soil material type, we ran multiple comparisons using student's t-test. Final results are displayed as the change in $\delta^{13}C$ values ($\Delta\delta^{13}C$) of each midge treatment compared to average of the control.

RESULTS

Microbial community composition

The relative abundance of soil microbial communities significantly changed after midge carcass addition (P=0.011, $F_{5,100}$ =2.07). The pressed treatment supported a different microbial community compared to the control and pulsed 2011 treatment (Fig. 1). Additionally, pairwise comparisons among all five treatments revealed not only a community shift from control levels in the pressed treatment (P=0.0035, $F_{1,61}$ =4.15), but also a significant difference between pressed and pulsed 2011 (P=0.0022, $F_{1,22}$ =5.25) communities. When broken down into sub-functional groups, pulsed treatments significantly decreased fungal abundance whereas pressed plots significantly increased both fungal and bacterial community abundance (Table 2).

Total soil microbial biomass was not significantly affected by midge carcass addition (P=0.097, $F_{5,103}$=1.92). However, both bacterial and fungal biomass was significantly higher in the pressed treatment (P<0.05). Fungal communities dominated the mineral soil and fungal biomass significantly decreased after a pulsed addition (Fig. 2). Across all treatments, the ratio of fungal to bacterial biomass did not significantly change in response to carcass addition (P=0.6871).

Microbial community function

Respired CO_2 from the organic top layer was higher than that respired from the mineral soil (Fig. 3) even though the rate of respired CO_2 overtime was statistically the same between the two soil layers (Table 3). In the organic top layer, respired CO_2 from the pressed and pulsed 2011 treatments significantly differed from the respired CO_2 released from the

control (P<0.001, Fig. 3, Table 3). In the mineral soil, respired CO_2 was unaffected in the pressed midge addition (P>0.05, Fig.3, Table 3) and positively affected in the pulsed treatment, suggesting that a single pulsed carcass addition was enough to affect the mineral soil even though a pressed addition had no effect. Over the course of the summer, the decomposition rate in the pulsed treatment converged on the same rate as in the controls in both soil layers. However, soil microbes in the pressed treatment decomposed organic material faster than both the control and pulse 2011 treatment (P>0.05, Fig.3, Table 3).

Stable isotopes

Relative to $\delta^{13}C$ values in the controls, the $\delta^{13}C$ of respired CO_2 was lower after pressed midge addition depending on the location within the soil profile and within midge carcass addition treatments (Fig.4). The relative difference in $\delta^{13}C$ values ($\Delta\delta^{13}C$) was significant for both soil layers and between both addition treatments (P<0.0001, $F_{3,92}$=59.71, Fig.4). The $\delta^{13}C$ of respired CO_2 in the organic top layer displayed lower $\delta^{13}C$ values across both treatments.

DISCUSSION:

Insects move across ecosystem boundaries and can provide neighboring ecosystems with external resources. Lakes, such as Lake Mývatn, that can support large populations of emergent insects may deliver otherwise unavailable nutrients to the surrounding terrestrial ecosystem. Our manipulative midge-addition study provides evidence of a strong direct bottom-up effect of aquatic insect deposition on terrestrial heathland systems. Midge carcass deposition onto land supplies a flux of organic nutrients to an otherwise nutrient limited

heathland and changes the composition of microbial communities and the rate in which microbes process organic matter. The few examples measuring relationships between carcass inputs and soil communities found that the combination of nutrients that carcasses provide (C, N, P), can reduce nutrient limitation and increase soil microbial biomass and CO_2 evolution overtime (Yang 2004, Carter et al. 2008). Similarly, Icelandic heathlands responded to midge carcasses addition through increased soil decomposition (rate of CO_2 flux overtime), increased microbial biomass, and overall community shifts where carcasses were added to the soil. Where lakes support large-scale insect emergence, we can anticipate increased decomposition and soil microbial community shifts in the near-shore heathland soil communities.

Similar to other studies, we found soil microbial community composition to be sensitive to nutrient addition (Allison and Martiny 2008). Pressed carcass addition had the largest lasting effect on soil microbial structure, supporting different relative abundance of communities and measurably higher total microbial biomass. The positive effects of continuous midge carcass addition complements other fertilization studies that have demonstrated a strong soil community response to continuous supply of fertilizer (Lavoie et al. 2011, Kallenbach and Grandy 2011). Contrary to our expectation that bacterial biomass would dominate areas receiving pressed carcass addition, both fungal and bacterial biomass benefited from pressed carcass inputs, with fungi dominating communities across all treatments. This result could be due to the accompanying changes in aboveground plant communities in midge addition plots (Hoekman *et al. in prep*), as complex plant-soil feedbacks can dictate beneficial fungal associations and overall microbial responses to nutrient addition (Aerts 1999, Ladygina and Hedlund 2010, Lavoie et al. 2011).

The pulsed treatment supported microbial communities with a different relative abundance, though no parallel treatment effects were measured in CO_2 flux overtime. This change in microbial community composition may not be apparent in the functionality of the soil, because multiple microbial species may fill the same functional role ("functional redundancy")(Nannipieri et al. 2003). Temperature, C content, soil pH, and atmospheric CO_2 are a few examples of abiotic factors that could interact with the soil community (Allison and Martiny 2008, Welc et al. 2012). Understanding the functional redundancy of these soil communities will require further research, as we did not monitor community composition under controlled abiotic conditions. However, we did find both a decrease in pulsed 2011 total microbial biomass and in mineral soil CO_2 flux, suggesting that lower overall microbial biomass (as opposed to the relative abundance of particular functional groups) may have a direct effect on the amount of CO_2 released from the soil.

Midge carcass addition played a strong role in mediating SOM decomposition in heathland ecosystems. Carcass addition affected CO_2 flux in both the organic layer and mineral soil. Since the organic top layer had more available organic matter than the mineral soil (Gudmundsson et al. 2004) the organic layer supported overall higher CO_2 flux. This was not surprising, as the deeper mineral soil tends to have larger stores of recalcitrant C compared to the surface soils. Due to higher accessibility of labile C on the surface, the top 0-5cm of the soil profile respires more CO_2 than the lower 5-15cm (Fierer et al. 2003). Though the total amount of CO_2 respired was different between the two soil layers in our study, the rate of CO_2flux overtime was the same, complementing other research that shows the rate of CO_2 flux overtime to change with nutrient addition in both mineral and organic soil layers in other heathland systems (Lavoie et al. 2011).

Measured differences in $\delta^{13}C$ values in the pressed treatment show that alongside increased microbial activity and abundance, communities were respiring a different form of C in response to midge addition. We expected to find a midge C signature in the CO_2 respired from our pressed treatments, but instead discovered that the microbial communities in the pressed treatments were likely consuming soil C. When C and N are added together, they can have a strong priming effect on the microbial communities (Milcu et al. 2011) by reducing the limitation of both C and N. It is possible that this priming effect allowed microbes in our midge addition treatments to access recalcitrant C already in the soil, though the limitations in our sampling procedure cannot provide direct evidence of this effect. Even so, microbial communities do consume a different form of C after pressed carcass addition.

We believe that while both fungal and bacterial communities responded after four years of carcass addition, the order in which these microbial communities responded depended on the timing of resource addition. Microbial community responses to initial resource addition (as shown in pulsed 2011) had lower overall community abundance, returning to control levels after one year of addition. Conversely, as resources were continually added (pressed), both fungal and bacterial community abundance increased. The delayed fungal response to carcass inputs over multiple years may be explained by the variation in consumption patterns of different microbial functional groups. Bacteria respond very quickly to labile carbon in the soil (Bardgett et al. 1995, Lovell et al. 1995, Tavi et al. 2013) and likely consumed midge carcasses as they became available. Though we saw a strong bacterial community increase in abundance and biomass in the pressed treatment, we did not see a change in bacterial biomass after a pulsed addition. It is possible that an immediate increase in bacterial communities may have occurred immediately following 2011

addition, but after resources were consumed, microbial communities experienced a "die off" effect due to overconsumption (Fig. 5). As fungi are important consumers of plant derived C (Tavi et al. 2013), the subsequent change in aboveground plant communities following bacteria-mediated nutrient turnover in pressed treatments could explain the significant increase in fungal abundance after four years of carcass addition.

Our research uniquely complements previous studies on the role of emergent insects in facilitating changes in ecosystem structure and function. Where large-scale insect movement has established influence over plant community composition (Raudenbush et al. *in prep*), arthropod community composition (Hoekman et al. 2011, Dreyer et al. 2012) and predator feeding habits (Paetzold et al. 2011, Akamatsu and Toda 2011), we may also see increased decomposition and microbial community shifts. Microbial communities directly used midge carcasses to increase biomass and shift community composition and increase decomposition rates and nutrient turnover. Since microbial communities mediate soil nutrient availability, soils receiving midge inputs may exert strong bottom–up control on aboveground plant communities. As soil microbial community structure and function changed in response to direct midge carcass addition, soil communities bordering lakes that produce large-scale insect emergence may be similarly affected by carcass deposition during emergence events.

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LITERATURE CITED

Aerts R. 1999. Interspecific competition in natural plant communities : mechanisms, trade-offs and plant–soil feedbacks. J Exp Bot 50:29–37.

Akamatsu F, Toda H. 2011. Aquatic subsidies transport anthropogenic nitrogen to riparian spiders. Environ Pollut 159:1390–7.

Allison S, Martiny J. 2008. Resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci 105:11512–9.

Anderson W, Polis G. 1999. Nutrient fluxes from water to land : seabirds affect plant nutrient status on Gulf of California islands. Oecologia:324–32.

Arnalds A. 2004. Carbon sequestration and the restoration of land health; and example from Iceland. Clim Change 65:333–46.

Arnalds O, Kimble J. 2001. Andisols of Deserts in Iceland. Soil Sci Soc Am J 65:1778–86.

Balser TC, Treseder KK, Ekenler M. 2005. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biol Biochem 37:601–4.

Bardgett RD, James L, Leemans DK. 1995. Microbial biomass and activity in a grassland soil amended with different application rates of silage effluent—A laboratory study. Bioresour Technol 52:175–80.

Bartrons M, Papeş M, Diebel MW, Gratton C, Zanden MJ. 2013. Regional-Level Inputs of Emergent Aquatic Insects from Water to Land. Ecosystems 16:1353–63.

Brehme CS, Boarman WI, Hathaway S a., Herring A, Lyren L, Mendelsohn M, Pease K, Rahn M, Rochester C, Stokes D, Turschak G, Fisher RN. 2009. Spatial and temporal patterns across an ecological boundary: Allochthonous effects of a young saltwater lake on a desert ecosystem. J Arid Environ 73:811–20.

Cusack DF, Silver WL, Torn MS, Burton SD, Firestone MK. 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. Ecology 92:621–32.

Demoling F, Ola Nilsson L, Bååth E. 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biol Biochem 40:370–9.

Dreyer J, Hoekman D, Gratton C. 2012. Lake-derived midges increase abundance of shoreline terrestrial arthropods via multiple trophic pathways. Oikos 121:252–8.

Esch EH, Hernández DL, Pasari JR, Kantor RSG, Selmants PC. 2012. Response of soil microbial activity to grazing, nitrogen deposition, and exotic cover in a serpentine grassland. Plant Soil 366:671–82.

Fagan WF, Siemann E, Mitter C, Denno RF, Huberty AF, Woods HA, Elser JJ. 2002. Nitrogen in insects: implications for trophic complexity and species diversification. Am Nat 160:784–802.

Fierer N, Schimel JP, Holden P a. 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol Biochem 35:167–76.

Frost C, Hunter M. 2004. Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. Ecology 85:3335–47.

Gratton C, Denno R. 2003. Inter-year carryover effects of a nutrient pulse on Spartina plants, herbivores, and natural enemies. Ecology 84:2692–707.

Gratton C, Donaldson J, Vander Zanden MJ. 2008. Ecosystem Linkages Between Lakes and the Surrounding Terrestrial Landscape in Northeast Iceland. Ecosystems 11:764–74.

Gratton C, Zanden M. 2009. Flux of aquatic insect productivity to land: comparison of lentic and lotic ecosystems. Ecology 90:2689–99.

Gudmundsson T, Björnsson H, Thorvaldsson G. 2004. Organic carbon accumulation and pH changes in an Andic Gleysol under a long-term fertilizer experiment in Iceland. Catena 56:213–24.

Hillstrom M, Meehan TD, Kelly K, Lindroth RL. 2010. Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO2 and O3. Plant Soil 336:75–85.

Hoekman D, Bartrons M, Gratton C. 2012. Ecosystem linkages revealed by experimental lake-derived isotope signal in heathland food webs. Oecologia 170:735–43.

Hoekman D, Dreyer J, Jackson RD, Townsend P a, Gratton C. 2011. Lake to land subsidies: experimental addition of aquatic insects increases terrestrial arthropod densities. Ecology 92:2063–72.

Jonsson M, Wardle D a. 2009. The influence of freshwater-lake subsidies on invertebrates occupying terrestrial vegetation. Acta Oecologica 35:698–704.

Kagata H, Ohgushi T. 2012. Non-additive effects of leaf litter and insect frass mixture on decomposition processes. Ecol Res 27:69–75.

Kallenbach C, Grandy a. S. 2011. Controls over soil microbial biomass responses to carbon amendments in agricultural systems: A meta-analysis. Agric Ecosyst Environ 144:241–52.

Krumins JA, Dighton J, Gray D, Franklin RB, Morin PJ, Roberts MS. 2009. Soil microbial community response to nitrogen enrichment in two scrub oak forests. For Ecol Manage 258:1383–90.

Ladygina N, Hedlund K. 2010. Plant species influence microbial diversity and carbon allocation in the rhizosphere. Soil Biol Biochem 42:162–8.

Lavoie M, Mack MC, Schuur E a. G. 2011. Effects of elevated nitrogen and temperature on carbon and nitrogen dynamics in Alaskan arctic and boreal soils. J Geophys Res 116:1–14.

Lennon JT, Cottingham KL. 2008. Microbial productivity in variable resource environments. Ecology 89:1001–14.

Leroux SJ, Loreau M. 2011. Dynamics of Reciprocal Pulsed Subsidies in Local and Meta-Ecosystems. Ecosystems 15:48–59.

Lovell RD, Jarvis SC, Bardgett RD. 1995. Soil microbial biomass and activity in long-term grassland: Effects of management changes. Soil Biol Biochem 27:969–75.

Lundberg J, Moberg F. 2003. Mobile link organisms and ecosystem functioning: implications for ecosystem resilience and management. Ecosystems 6:87–98.

Malison RL, Baxter C V. 2010. The fire pulse: wildfire stimulates flux of aquatic prey to terrestrial habitats driving increases in riparian consumers. Can J Fish Aquat Sci 67:570–9.

Milcu A, Heim A, Ellis RJ, Scheu S, Manning P. 2011. Identification of General Patterns of Nutrient and Labile Carbon Control on Soil Carbon Dynamics Across a Successional Gradient. Ecosystems 14:710–9.

Moore JC, Berlow EL, Coleman DC, Ruiter PC, Dong Q, Hastings A, Johnson NC, McCann KS, Melville K, Morin PJ, Nadelhoffer K, Rosemond AD, Post DM, Sabo JL, Scow KM, Vanni MJ, Wall DH. 2004. Detritus, trophic dynamics and biodiversity. Ecol Lett 7:584–600.

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G. 2003. Microbial diversity and soil functions. Eur J Soil Sci 54:655–70.

Nilsson LO, Wallander H, Gundersen P. 2011. Changes in microbial activities and biomasses over a forest floor gradient in C-to-N ratio. Plant Soil 355:75–86.

Nowinski NS, Trumbore SE, Schuur E a. G, Mack MC, Shaver GR. 2008. Nutrient Addition Prompts Rapid Destabilization of Organic Matter in an Arctic Tundra Ecosystem. Ecosystems 11:16–25.

Nowlin W, Vanni M, Yang L. 2008. Comparing resource pulses in aquatic and terrestrial ecosystems. Ecology 89:647–59.

Óskarsson H, Arnalds Ó, Gudmundsson J, Gudbergsson G. 2004. Organic carbon in Icelandic Andosols: geographical variation and impact of erosion. Catena 56:225–38.

Paetzold A, Smith M, Warren PH, Maltby L. 2011. Environmental impact propagated by cross-system subsidy: Chronic stream pollution controls riparian spider populations. Ecology 92:1711–6.

Rumpel C, Rodríguez-Rodríguez a., González-Pérez J a., Arbelo C, Chabbi a., Nunan N, González-Vila FJ. 2011. Contrasting composition of free and mineral-bound organic matter in top- and subsoil horizons of Andosols. Biol Fertil Soils 48:401–11.

Rundio DE, Lindley ST. 2012. Reciprocal fluxes of stream and riparian invertebrates in a coastal California basin with Mediterranean climate. Ecol Res 27:539–50.

Schwinning S, Sala OE. 2004. Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. Oecologia 141:211–20.

Stark S, Eskelinen A, Männistö MK. 2011. Regulation of Microbial Community Composition and Activity by Soil Nutrient Availability, Soil pH, and Herbivory in the Tundra. Ecosystems 15:18–33.

Strickland MS, Rousk J. 2010. Considering fungal: bacterial dominance in soils – Methods, controls, and ecosystem implications. Soil Biol Biochem 42:1385–95.

Tavi NM, Martikainen PJ, Lokko K, Kontro M, Wild B, Richter A, Biasi C. 2013. Linking microbial community structure and allocation of plant-derived carbon in an organic agricultural soil using 13CO2 pulse-chase labelling combined with 13C-PLFA profiling. Soil Biol Biochem 58:207–15.

Vanderwal A, Vanveen J, Pijl A, Summerbell R, Deboer W. 2006. Constraints on development of fungal biomass and decomposition processes during restoration of arable sandy soils. Soil Biol Biochem

Welc M, Bünemann EK, Fließbach A, Frossard E, Jansa J. 2012. Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. Soil Biol Biochem 49:184–92.

Yang L. 2004. Periodical Cicadas as Resource Pulses in North American Forests. Science 306:1565–7.

Yee D a, Juliano S a. 2012. Concurrent effects of resource pulse amount, type, and frequency on community and population properties of consumers in detritus-based systems. Oecologia 169:511–22.

Zelles L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil : a review:111–29.

TABLES:

Table 1: Categorical listing of microbial groups sampled from heathland andosol soils, identified by lipid C chain length. Groups broadly define whether a given species is a fungi or bacteria. Fungi species are bolded.

FA's	Biomarker	Group
12:00	Bacteria	Bacteria
13:00	Bacteria	Bacteria
14:00	Bacteria	Bacteria
15:00	Bacteria	Bacteria
16:00	Bacteria	Bacteria
17:00	Bacteria	Bacteria
18:00	Bacteria	Bacteria
19:00	Bacteria	Bacteria
12:0 3OH	gm-	Bacteria
13:0 ANTEISO	gm+	Bacteria
13:0 ISO	gm+	Bacteria
14:0 ISO	gm+	Bacteria
14:1 w6c	gm-	Bacteria
15:0 ANTEISO	gm+	Bacteria
15:0 ISO	gm+	Bacteria
15:1 w8c	Bacteria	Bacteria
16:0 10 Methyl	actinomycete	Bacteria
16:0 2OH	gm-	Bacteria
16:0 3OH	gm-	Bacteria
16:0 ISO	gm+	Bacteria
16:1 w5c	**AMF**	**Fungi**
16:1 w7c	gm-	Bacteria
16:1 w9c	gm-	Bacteria
17:0 10 Methyl	actinomycete	Bacteria
17:0 ANTEISO	gm+	Bacteria
17:0 CYCLO	gm- and anerobe	Bacteria
17:0 ISO	gm+	Bacteria
17:1 w7c	gm-	Bacteria
17:1 w8c	gm-	Bacteria
18:0 10 Methyl	actinomycete	Bacteria
18:1 2OH	gm-	Bacteria
18:1 w7c	gm-	Bacteria
18:1 w9c	**fungi (sf or ecto)**	**Fungi**
18:2 w6,9c	**sapro fungi**	**Fungi**
18:3 w6,9,12c	**sapro or protozoa**	**Fungi**
19:0 10 Methyl	actinomycete	Bacteria
19:0 CYCLO	gm- and anerobe	Bacteria

Table 2: Relative abundance (mol% species/total species) of heathland andosol microbial communities within each midge addition treatment. Some lipids could only be identified as bacteria or fungi while others were identified to sub functional group. Gm+ bacteria exclude actinomycetes. Bolded values with an asterix (*) indicate significant treatment differences compared to the control for each group ($p<0.05$).

Treatment	*Total species abundance*	*Bacteria (total)*	actinomycete	gm+	gm-	*Fungi (total)*	AMF	saprophytic
Press	**3.53 ***	**2.65 ***	0.16	**0.55 ***	0.75	**0.89 ***	**0.13 ***	0.44
Pulse 2011	**2.99 ***	2.27	0.11	0.39	0.61	**0.72 ***	**0.09 ***	**0.38 ***
Pulse 2010	3.23	2.41	0.13	0.44	0.67	0.82	0.13	0.42
Pulse 2009	3.32	2.46	0.15	0.48	0.69	**0.87 ***	0.13	**0.46 ***
Pulse 2008	3.35	2.48	0.14	0.45	0.70	**0.87 ***	0.12	0.44
Control	3.24	2.43	0.13	0.44	0.68	0.81	0.12	0.42

Table 3: Values display the natural log of respired CO_2 (ug C g^{-1} material d^{-1}) and respired CO_2 overtime (decomposition rate) from two different heathland soil layers during summer of 2011. Values and standard error were rounded to the nearest hundredth. Asterix (*) indicates a significant midge treatment effect compared to control (P<0.05).

Soil Layer	Treatment	Respired C	Respired C overtime
Organic top layer	Press	**6.87 ± 0.18 ***	**-0.06 ± 0.01 ***
	Pulse 2011	**6.68 ± 0.21 ***	-0.05 ± 0.01
	Control	6.20 ± 0.07	-0.04 ± 0.01
Mineral soil	Press	4.13 ± 0.44	**-0.06 ± 0.01 ***
	Pulse 2011	**3.44 ± 0.52 ***	-0.05 ± 0.01
	Control	3.65 ± 0.17	-0.04 ± 0.01

FIGURES:

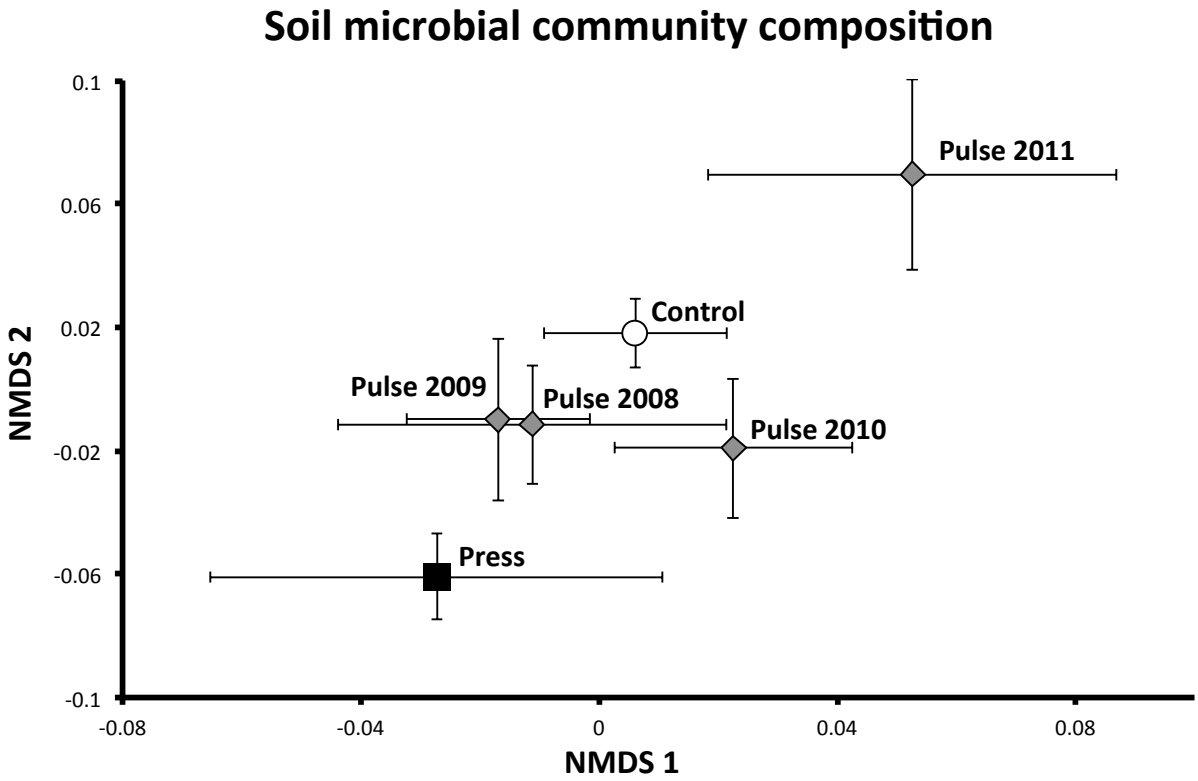

Figure 1: Treatment effects on heathland soil microbial community composition. Microbial communities in the pressed treatment were overall more abundant compared to the pulsed and control treatments. NMDS accounted for 91% of the variability in community data along axis 1 and 2. Error bars are 1 std error from the mean.

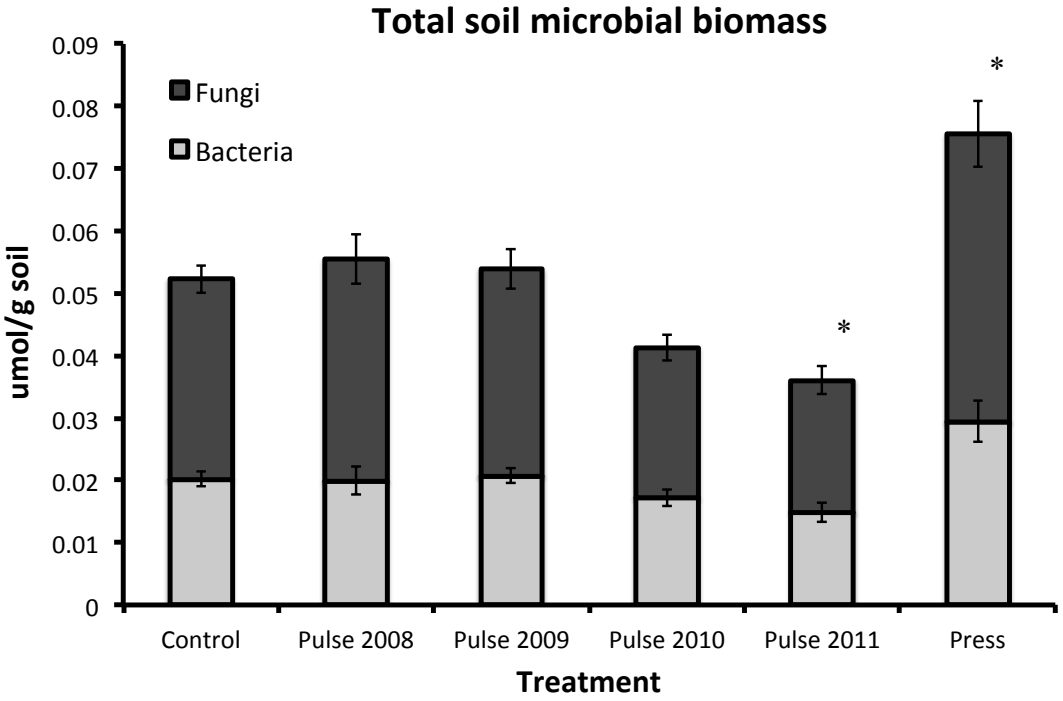

Figure 2: Treatment effects on total soil microbial community biomass in heathland mineral soil. Stacked bars show midge treatment effects on bacterial and fungal biomass. The ratio of fungi:bacteria did not significantly change among treatments. Error bars are 1 std error from the mean. Asterix (*) indicates a significant midge treatment effect compared to control (P<0.05).

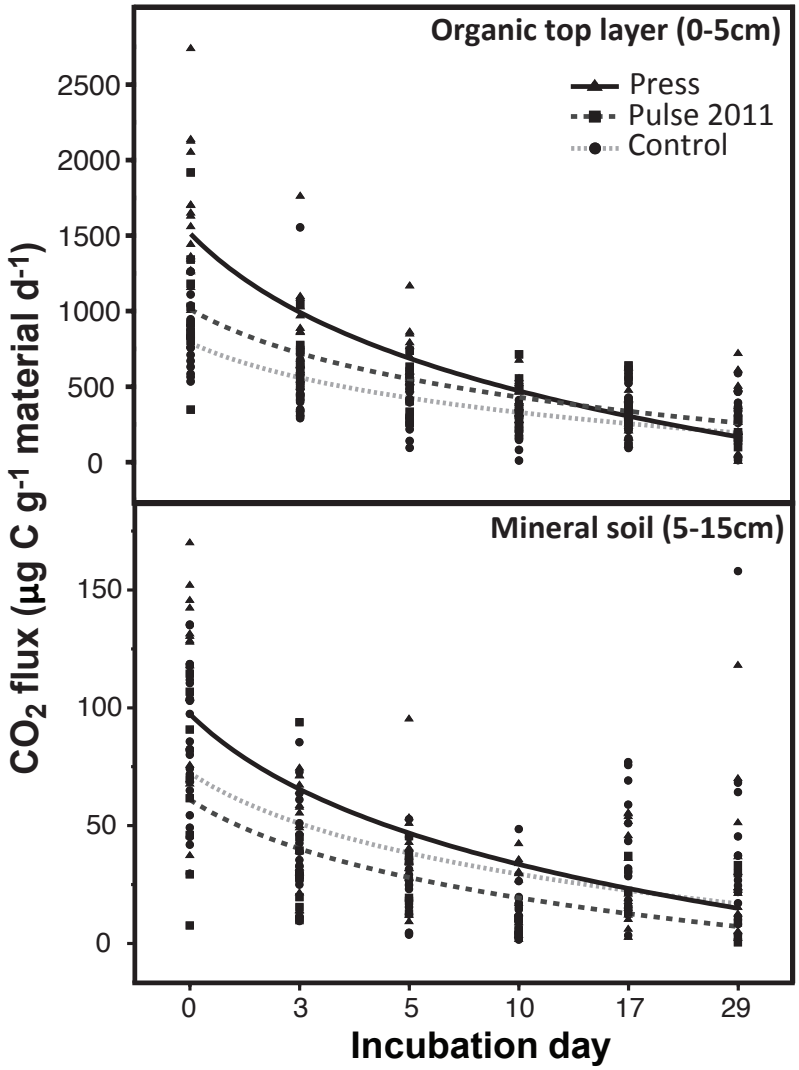

Figure 3: Measurements of respired CO_2 flux (ug C g^{-1} material d^{-1}) and respired CO_2 overtime taken from heathland soils treated with a pressed or pulsed midge addition treatment in 2011. Each line represents a midge addition treatment. Y-axis values are different among soil layer types. The organic top layer had the highest overall respired CO_2 flux compared to all other panels.

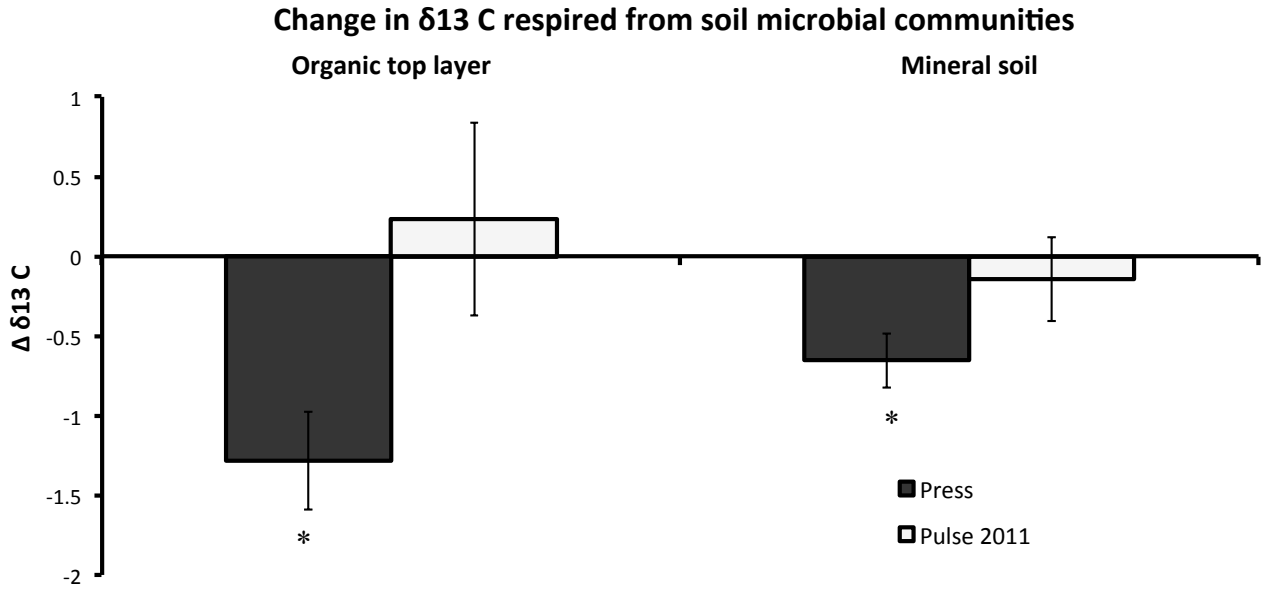

Figure 4: Measured changes from control levels in $d^{13}C$ signatures from microbial respired CO_2 from soil sampled from pressed and pulsed treatment plots. Changes were also measured between the two soil layers (organic top layer and mineral soil). Asterix (*) indicates a significant midge treatment effect compared to control ($P<0.05$). The type of C consumed by microbial communities changed the most in pressed treatments.

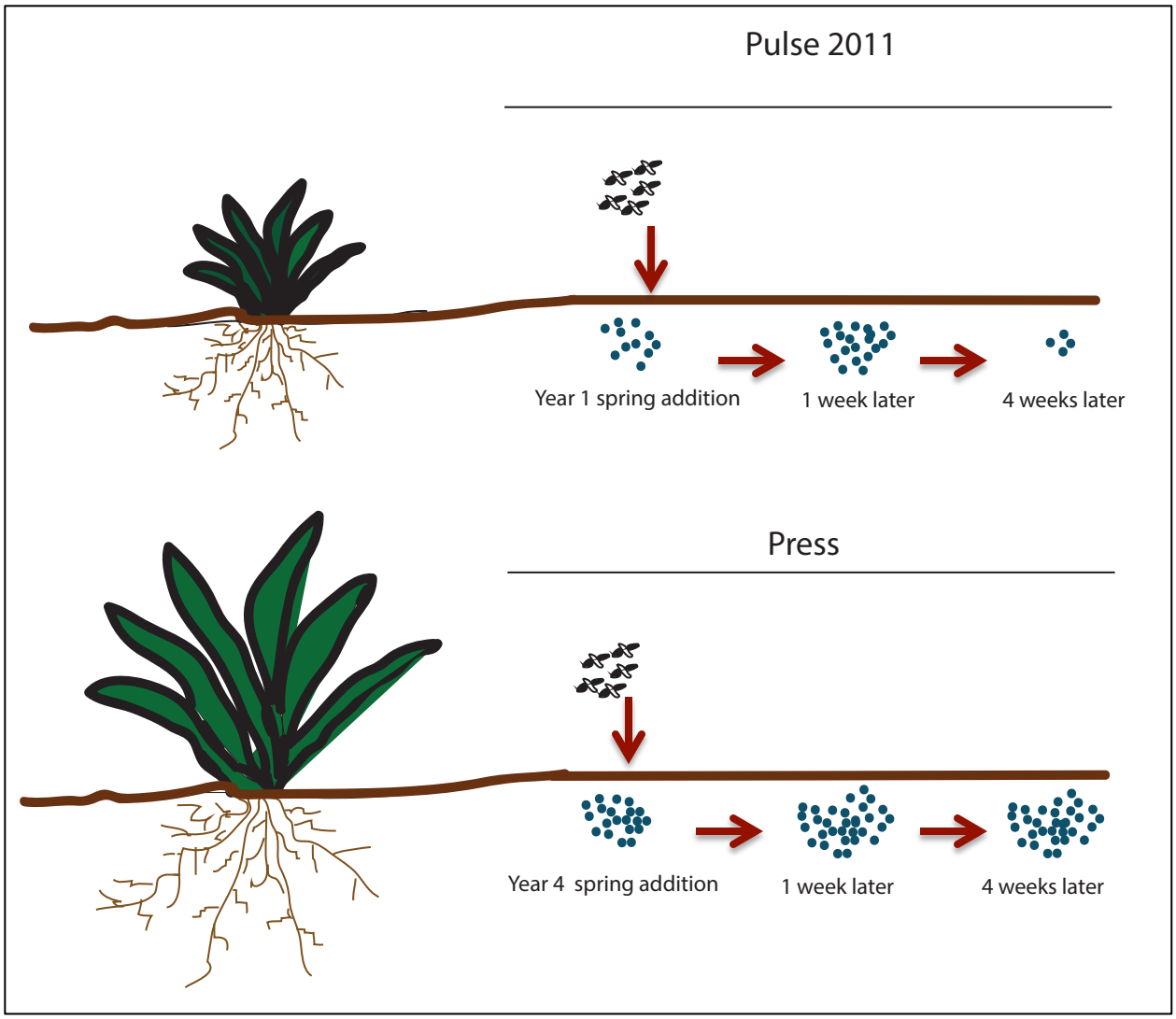

Figure 5: A conceptual diagram showing the "die off" effect of microbial communities immediately following a nutrient addition. The microbial community response to a single year addition (pulse) is different after four years worth of addition (press).