

Ecosystem linkages revealed by experimental lake-derived isotope signal in heathland food webs

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Abstract Cross-ecosystem movement of nutrients and biomass can have important effects on recipient systems. Emerging aquatic insects are subsidies to terrestrial ecosystems and can influence foodweb interactions in riparian systems. In a 2-year field experiment, we simulated aquatic insect deposition by adding adult midge carcasses (150 g dry mass m^{-2} year $^{-1}$) to 1- m^2 heathland plots at a site with low natural midge deposition. We established four levels of midge-addition treatments and measured stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in plants and arthropods within each treatment. We used a multiple-source isotope Bayesian mixing model to estimate the terrestrial versus aquatic contribution to the diets of arthropods. Aquatic resources were incorporated into plant, detritivore, and predator biomass. Detritivorous Collembola showed the greatest difference in isotope values (+3 ‰ $\delta^{15}N$ and +4 ‰ $\delta^{13}C$) between midge-addition and reference treatments. Isotope values of small spiders followed the same trend of enrichment as Collembola while other arthropods (mites and large spiders) were only enriched after 2 years of midge addition. Although predator diets did not change,

they became isotopically enriched via their likely prey (Collembola). Plants also had elevated $\delta^{15}N$ (+1 ‰) in midge-addition treatments. The time required and amount of midge-derived C and N detected varied and depended on trophic position. Midge-derived nutrients were no longer present in arthropod biomass in the year following midge addition. Aquatic insect carcasses can be rapidly incorporated into terrestrial food webs in nearshore habitats, and repeated inputs can be detected at multiple trophic levels, thus highlighting the importance of the detrital pathway for aquatic to terrestrial cross-ecosystem subsidies.

Keywords Aquatic–terrestrial linkage · Arthropod food web · Cross-ecosystem subsidy · Resource pulse · Stable isotopes

Introduction

Food-web linkages between adjacent ecosystems have important consequences for the dynamics of recipient systems (Polis et al. 1997). When resources cross ecosystem boundaries, less productive systems can potentially be subsidized by more productive systems (Gravel et al. 2010). For example, unproductive gravel river banks (Paetzold et al. 2005) and isolated oceanic islands (Spiller et al. 2010) can support larger plant and animal populations as a result of allochthonous resources they receive from nearby productive aquatic systems. The connection between lakes and surrounding terrestrial areas, however, is a less studied aquatic–terrestrial linkage, but accumulating evidence suggests this connection is also important (Gratton et al. 2008; Jonsson and Wardle 2009; Dreyer et al. 2012; Hoekman et al. 2011). Lakes can support large populations of larval aquatic insects, which in their adult

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stages move over land and thus transport biomass and nutrients to the adjacent terrestrial systems (Gratton and Vander Zanden 2009). These subsidies can influence lakeside terrestrial food webs, but the important trophic pathways and temporal scale of aquatic resource transfer into the terrestrial food web are largely unknown. Moreover, effects of allochthonous inputs on food webs are often difficult to disentangle from other environmental gradients that exist at the land–water boundary (Richardson et al. 2010).

The land–water ecotone is characterized by resource exchange that often occurs in pulses and can have demonstrable effects on recipient food webs (Yang et al. 2008). Historically, most cross-ecosystem studies at the land–water ecotone have focused on movement into aquatic systems, though recently this boundary has been recognized for its reciprocal subsidies (Nakano and Murakami 2001; Leroux and Loreau 2011). Animal movement is often responsible for nutrient pulses from aquatic systems that affect terrestrial food webs, with salmon carcasses in riparian areas being a prime example (Hocking et al. 2009). Aquatic insects can also represent nutrient pulses to land because aquatic insects often emerge synchronously from lakes and streams and move over land to mate. Lakeshore areas can support higher densities of arthropods than areas farther from lakes (Jonsson and Wardle 2009; Dreyer et al. 2012) and isotopic evidence suggests riparian arthropods utilize lake-derived resources (Gratton et al. 2008). Similar patterns of higher densities (Power et al. 2004) and isotopically enriched animals (Paetzold et al. 2005) have been documented in riparian areas along streams compared to farther inland. In addition, water-to-land resource pulses vary in magnitude across time. The abundance of aquatic insects entering a terrestrial ecosystem differs between species and generations within a year and between years. Variation in the frequency and magnitude of resource pulses may have important consequences for food webs in recipient systems (Yang et al. 2008).

Lake Mývatn (“lake of the midges” in Icelandic) is a large productive lake in northeast Iceland (Einarsson et al. 2004), known for extremely high densities of midges (Diptera: Chironomidae) that live in the shallow benthos throughout the lake (Lindegaard and Jonasson 1979). Midges emerge from the lake and form dense swarms of millions of individuals within 150 m of the shoreline (hereafter “nearshore”). Although a fraction of the females return to the lake to deposit eggs, many midges die in the nearshore environment and their biomass enters the terrestrial food web as prey for predators, or resources for decomposers and plants. Their relative abundance varies between years by four orders of magnitude and thus fluctuates from high- to low-midge years on 5- to 7-year cycles

(Einarsson et al. 2002). The cyclic and pulse-like nature of midge emergences raises questions of how much aquatic resources affect terrestrial food webs and how these effects change over time.

Dreyer et al. (2012) sampled terrestrial arthropods at this lake and others in northeast Iceland that varied in aquatic insect density and found higher densities of terrestrial predators and detritivores near lakes with higher aquatic insect density. At the same lakes, Gratton et al. (2008) found that some arthropod predators and detritivores near lakes with large insect emergences were enriched in $\delta^{13}\text{C}$ relative to arthropods from lakes lacking insect emergences, suggesting reliance on aquatic resources. However, both these studies were observational, taking advantage of natural gradients of insect deposition around a suite of lakes and examining the concomitant responses of arthropod abundance and isotopic values. A limitation of observational studies is the potential to ascribe causation to spatially confounded variables. To address this, we performed an experiment to study the effects of aquatic resources on population densities (Hoekman et al. 2011) and isotopic values of terrestrial food webs by simulating midge deposition to a terrestrial system that lacks substantial aquatic insect input. To simulate midge deposition into the terrestrial food web, we added dead midges to plots adjacent to a low-midge lake, Helluvaðstjörn. Natural aquatic insect emergence and deposition on land at Helluvaðstjörn are very low ($<0.5 \text{ g m}^{-2} \text{ year}^{-1}$) compared to the high-midge Lake Mývatn ($>20 \text{ g m}^{-2} \text{ year}^{-1}$) (dry mass midge infall on land within 500 m of shore in 2008 and 2009; Dreyer et al., unpublished data). Land within 50 m of shore at Mývatn can receive $500\text{--}1,500 \text{ g m}^{-2} \text{ year}^{-1}$ in high-midge years (Dreyer et al., unpublished data). The experimental design of this study allowed us to simulate midge deposition and measure the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in terrestrial plant and arthropod biomass in midge-addition and control plots for 2 years while controlling for other potentially confounding variables.

We expected that experimental midge deposition would increase terrestrial productivity in our plots as midge C and nutrients became incorporated into the terrestrial food web. We hypothesized that first microbes, followed by arthropod detritivores, and eventually plants and arthropod predators would acquire midge-derived resources. We predicted that midge incorporation into the terrestrial arthropod food web would be revealed by enriched isotopic signatures of organisms in midge-addition plots, that 2 years of midge addition would result in more isotopic enrichment in terrestrial organisms than a single-year addition, and that the midge isotopic signature would be strongest in detritivores that can directly consume added detrital resources. In addition, we predicted that organisms further removed from the midge subsidy, either predators that consume

detritivores or plants that access midge nutrients after their release into the soil, would show a lagged response to midge-derived isotopes in their tissues.

Materials and methods

Experimental set-up and treatment application

We established experimental midge-addition plots at Helluvaðstjörn, a small (800 × 400 m) lake in northern Iceland (310 m above sea level, 1.2 m depth) (Lawson et al. 2007). A 700-m² experimental area with flat topography and homogeneous vegetation 200 m from the shore was fenced off to exclude sheep from grazing. Vegetation at the site is dominated by heathland species, including ericaceous shrubs *Betula nana*, *Arctostaphylos uva-ursi*, *Calluna vulgaris*, *Salix lanata*, and *Salix phylicifolia*. Grasses and forbs are also present at low densities. In 2008, we marked 16 pairs of 1-m² open plots (spaced 0.5–2 m apart) within this area. Paired plots were as similar as possible in vegetation composition by visual inspection. Each pair was randomly assigned to be either a midge-addition or a control (reference) plot (Appendix A in ESM).

Live adult midges were collected with sweep nets at Lake Mývatn during midge emergences (see Hoekman et al. 2011 for details). Mývatn is a large eutrophic lake 6 km east of Helluvaðstjörn, but with midge densities >50 times higher (Einarsson et al. 2004). Midges from Mývatn are approximately 50 % C and 10 % N, and the assemblage is comprised primarily of two species, *Tanytarsus gracilentus* (80 %) and *Chironomus islandicus* (15 %). Midges were frozen, dried, and weighed into 50-g aliquots. Midge addition plots received 50 g of dry midge carcasses, sprinkled evenly by hand 3 times during the summer (late May/early June, late June/early July, late July) at 150 g m⁻² (75 g C and 15 g N m⁻²) total. This reasonably approximates midge deposition in nearshore areas at Mývatn.

In 2009, four treatment combinations were established (Appendix A in ESM). Plots to which midges had not been added in 2008 were left unmanipulated and remained as “controls” in 2009 ($n = 32$). Half the plots that received midges in 2008 also received midges in 2009 and are called 2-year midge-addition or “press” plots ($n = 16$). The other half of the plots that received midges ($n = 16$) in 2008 did not receive any midge additions in 2009 and were termed the “pulse-08” plots. In addition, we added 16 new unpaired plots close to our 2008 plots and termed them “pulse-09” plots. Dried midges were added to the pulse-09 and press plots in the summer of 2009 using the same methodology as in 2008.

Isotope sample collection and preparation

To estimate the incorporation of midge-derived resources into the food web, we collected terrestrial arthropods and plants for analysis of stable isotope ratios of C and N. In both years, we sampled the arthropod communities in early August by vacuum sampling (SH 85 Shredder Vac/Blower; Stihl, USA). In each plot, the vacuum sampler was pressed over the ground to remove the arthropods from the vegetation and leaf litter. The contents of each vacuum bag were emptied into white plastic trays in the field and arthropods were removed using aspirators. Arthropod samples were frozen, dried, and sorted into the following taxonomic groups: Lycosidae, Gnaphosidae, Linyphiidae, Collembola, Acari, Hymenoptera, and Diptera larvae. These taxa represent dominant functional groups and were relatively abundant (see also Hoekman et al. 2011) so we could obtain sufficient biomass for isotope assays. Live leaf tissue was also collected from *Betula* to *Arctostaphylos* in 2009. Samples were dried and weighed into tin capsules for isotope analysis. Most arthropods were placed whole into the capsules (singly or bulked to achieve a minimum mass for analysis), although plants and large spiders (>1.5 mg) were ground to powder to homogenize their tissues before being weighed.

Samples were analyzed for stable isotopes of C and N using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK) at the Davis Stable Isotope Facility (University of California, Davis, CA, USA). Ratios of ¹³C/¹²C and ¹⁵N/¹⁴N are expressed relative to known standards [Vienna PeeDee Belemnite (VPDB) and atmospheric N, respectively] in per mil (‰) notation. Measurement errors (standard deviation) on the laboratory standards for $\delta^{15}\text{N}$ were 0.05 and 0.15 and for $\delta^{13}\text{C}$ were 0.20 and 0.13 for plants and arthropods, respectively. The standard deviation of internal replicates for $\delta^{15}\text{N}$ was 0.04 and 0.20 and for $\delta^{13}\text{C}$ were 0.11 and 0.37 for plants and arthropods, respectively.

Natural stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) have been used to study aquatic-terrestrial food web linkages (Collier et al. 2002; Paetzold et al. 2006) and present a time-integrated measure of fractional nutrient accumulation in a consumer. Thus, a one-time sample of plants and arthropods at the peak of the growing season (early August) is a good isotopic representation of prior resource use in the experimental plots. Carbon fixed by terrestrial plants has a $\delta^{13}\text{C}$ value around -27 ‰, while aquatic primary producers are more enriched and have $\delta^{13}\text{C}$ values around -13 ‰ at Mývatn. The C signature of consumers is determined by the C source at the base of the food web, and therefore the $\delta^{13}\text{C}$ value of a consumer is an indicator of whether it was feeding on terrestrial versus aquatic food sources. Because

midges feed and grow exclusively in the water, their $\delta^{13}\text{C}$ value is distinct and highly enriched compared to arthropods feeding on terrestrial sources. Stable N isotopes are also useful in food web studies because $\delta^{15}\text{N}$ values increase with each trophic transfer, and are therefore often used to estimate trophic position (Minagawa and Wada 1984).

Statistical methods

A MANOVA was used to test for differences among midge-addition treatments in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of plant and arthropod biomass. Variation in arthropod and plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were further analyzed by one-way ANOVA separately for each year with treatment as a fixed effect for each taxonomic group (2008: 2008 control, midge addition; 2009: 2009 control, pulse-08, pulse-09, press). A priori contrasts between each midge-addition treatment and the appropriate control (2008 or 2009) were performed. MANOVA, ANOVA and contrasts were conducted in JMP v.9 (SAS Institute, 2010).

Mixing models

Multiple-source Bayesian mixing models were used to infer diet changes of terrestrial arthropods under the different midge addition treatments. Only arthropods present in all treatments were included in the analysis (Appendix B in ESM). Isotope sources (N and C isotope means and standard deviations) for Collembola were plants and midges, while sources for predators, Lycosids, Linyphiids, and Acari, were Collembola, midges, and all other predators. Although Acari are a heterogeneous feeding group and can span from detritivorous to predatory (Walter and Proctor 1999), they were considered in the predator functional group in this analysis because their $\delta^{15}\text{N}$ values were comparable to the spiders. For the detritivorous Collembola, potential sources were limited to midges and plants (*Arctostaphylos* and *Betula*). Although these detritivores do not consume plants directly and are more likely associated with microbes decomposing decaying plants and litter (Hopkin 1997), the plant isotope sources are reasonable representations of one isotopic source consumed for this group (Gratton and Denno 2006; Gratton et al. 2008). Collembola are also known decomposers of dead animals. Midges were included in all models as an end member (even though they were not added to all treatments) so that their influence could be equally accounted across treatments.

Two different mixing models were fit for each taxon. First, we determined the potential diet composition for each consumer based on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of their potential prey within the *same* treatment as the assayed

consumer and the aquatic isotope source (midges). This “Direct midge consumption” model accounts for the possibility that all potential resources become enriched in midge-addition plots. A second model was fit for each arthropod consumer in a given treatment using the isotopic values of potential sources (prey) measured in *control* plots irrespective of the treatment from which the consumer was sampled. This model was termed the “Direct and indirect consumption” model because it places all possible conduits of midge-derived isotopes, both direct consumption of dead midges and consumption of prey that had fed on midges (i.e., indirect pathways), into the “midge” source component of the model. The difference between the two models reflects the degree to which midges are consumed either directly or indirectly through enriched prey. This contrast is most relevant for predators since Collembola’s isotopic resources are virtually unchanged in the formulations of the two models (i.e., plants change very little in the midge addition plots).

Multiple-source Bayesian mixing models were used to fit isotope data under the two different approaches outlined above using the SIAR 4.0.2 package (Moore and Semmens 2008; Jackson et al. 2009) in R (R Development Core Team 2009). Inputs to the models were N and C isotope means and standard deviations for the sources. We incorporated trophic discrimination factors ($3 \pm 1\%$) (Oelbermann and Scheu 2002) as a prior probability distribution for $\delta^{15}\text{N}$ (Galvan et al. 2012). No trophic fractionation was assumed for $\delta^{13}\text{C}$ (Gratton and Denno 2006). Mixing models were fit for each major consumer separately in each midge addition treatment using isotope data from 2009 only since plant isotopes were unavailable for 2008. By iterating model fits 10,000 times, the model estimated the posterior probability distribution of a particular diet source. Diet sources were considered to be different between midge-addition and control treatments if their 97.5 % credibility intervals did not overlap (Polito et al. 2011).

Results

Arthropods in the control treatment (with no midges added) had depleted $\delta^{13}\text{C}$ values with a narrow average range (-27 to -23%). Functional groups of plants, detritivores and predators could be differentiated along an axis of increasing $\delta^{15}\text{N}$ (Fig. 1a). Large-bodied spiders, Lycosidae and Gnaphosidae, had higher $\delta^{15}\text{N}$ than small-bodied predators, Linyphiidae and Acari. Midges were enriched in $\delta^{13}\text{C}$ (-14%) compared to terrestrial organisms. In the midge-addition plots (Fig. 1b), the functional groups were arranged similarly in isotopic space but shifted toward higher C and N isotopic values. Parasitic Hymenoptera and their detritivorous Diptera larvae hosts were abundant in

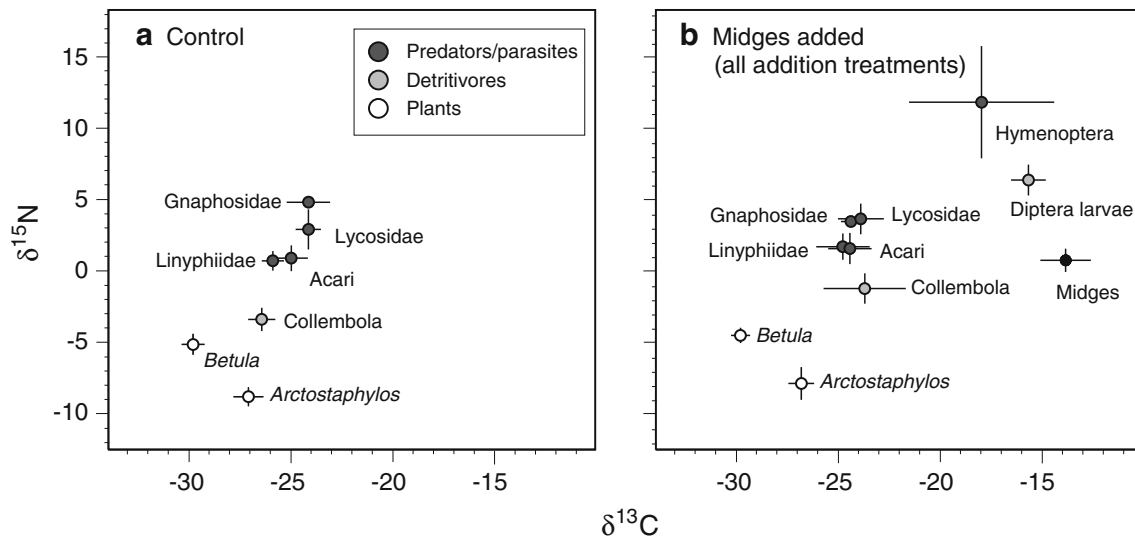


Fig. 1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) for plants and arthropod detritivores, predators and parasites collected from **a** control or **b** midge-addition experimental plots in 2008 and 2009. In 2009,

midge-addition includes pulse-08, pulse-09 and press. The isotopic value of midges added to midge-addition plots is noted as a reference point

midge-addition plots and were highly enriched in ^{13}C and ^{15}N compared to other members of the food web.

Arthropod detritivores readily assimilated midge-derived C and N into their bodies. To assess the degree of reliance on midge-derived resources, we calculated the difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the midge-addition and control treatments. In 2008, midge addition significantly increased the isotopic values of Collembola by +4 and +3 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (MANOVA exact $F_{2,4} = 28.3$, $P = 0.004$). No other taxon showed significant differences between control and midge-addition treatments in 2008, due to extremely small sample sizes ($n = 2\text{--}5$, Appendix B in ESM, full MANOVA results in Appendix C in ESM). The magnitude of the response was similar between years, but the sampling effort was higher in 2009. In 2009, organisms in midge-addition plots were enriched in both ^{13}C and ^{15}N (Fig. 2). Isotope values in midge-addition and control plots were significantly different for all groups ($P < 0.03$, Appendix C in ESM). As in the prior year, Collembola responded most strongly to midge-addition treatments with changes of about +4 and +2.5 ‰ in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively, in the pulse-09 and press treatments relative to controls (Fig. 2a). Collembola isotope values measured in the year after midge addition (pulse-08 treatment) showed only slightly elevated $\delta^{15}\text{N}$ (+1 ‰) but no change in $\delta^{13}\text{C}$ (Appendix D in ESM).

In addition to detritivores, midge-derived resources were also detected in predator biomass. The two main spider taxa, Linyphiidae and Lycosidae, were enriched in ^{13}C and

^{15}N in midge-addition plots (Fig. 2b, c) in 2009. Linyphiids had elevated $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the press and pulse-09 treatments and enriched $\delta^{15}\text{N}$ in the pulse-08 treatment (Fig. 2b). Lycosids had elevated $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the press treatment and elevated $\delta^{15}\text{N}$ values in the pulse-09 treatment (Fig. 2c), but isotope values were no different than controls in the year after midge additions (i.e., pulse-08, Appendix D in ESM). Acari (mites) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were enriched only in the 2-year press treatment (Fig. 2d; Appendix D in ESM).

Plants also incorporated midge-derived nutrients. The ericaceous shrub *Arctostaphylos* was enriched in $\delta^{15}\text{N}$ in the year following a midge addition (Fig. 2e, Appendix D). In contrast, *Betula* was enriched in $\delta^{15}\text{N}$ in the press and pulse-09 treatments but did not show a response to midge additions from the prior year (Fig. 2f). *Betula* $\delta^{13}\text{C}$ remained unchanged during the course of this experiment.

Mixing models analysis

The contribution of midges to Collembola diets greatly increased in 2-year press and the pulse-09 treatments (20 and 18 %, respectively), relative to controls (3 %, Table 1) according to the “direct midge consumption” Bayesian mixing model, but did not differ for other groups. In contrast, the “direct and indirect midge consumption” Bayesian mixing model estimated that for all taxa the percentage of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ incorporated into consumers due to a midge contribution increased from the control to 2-year press treatment (Collembola 6–8 %, mites

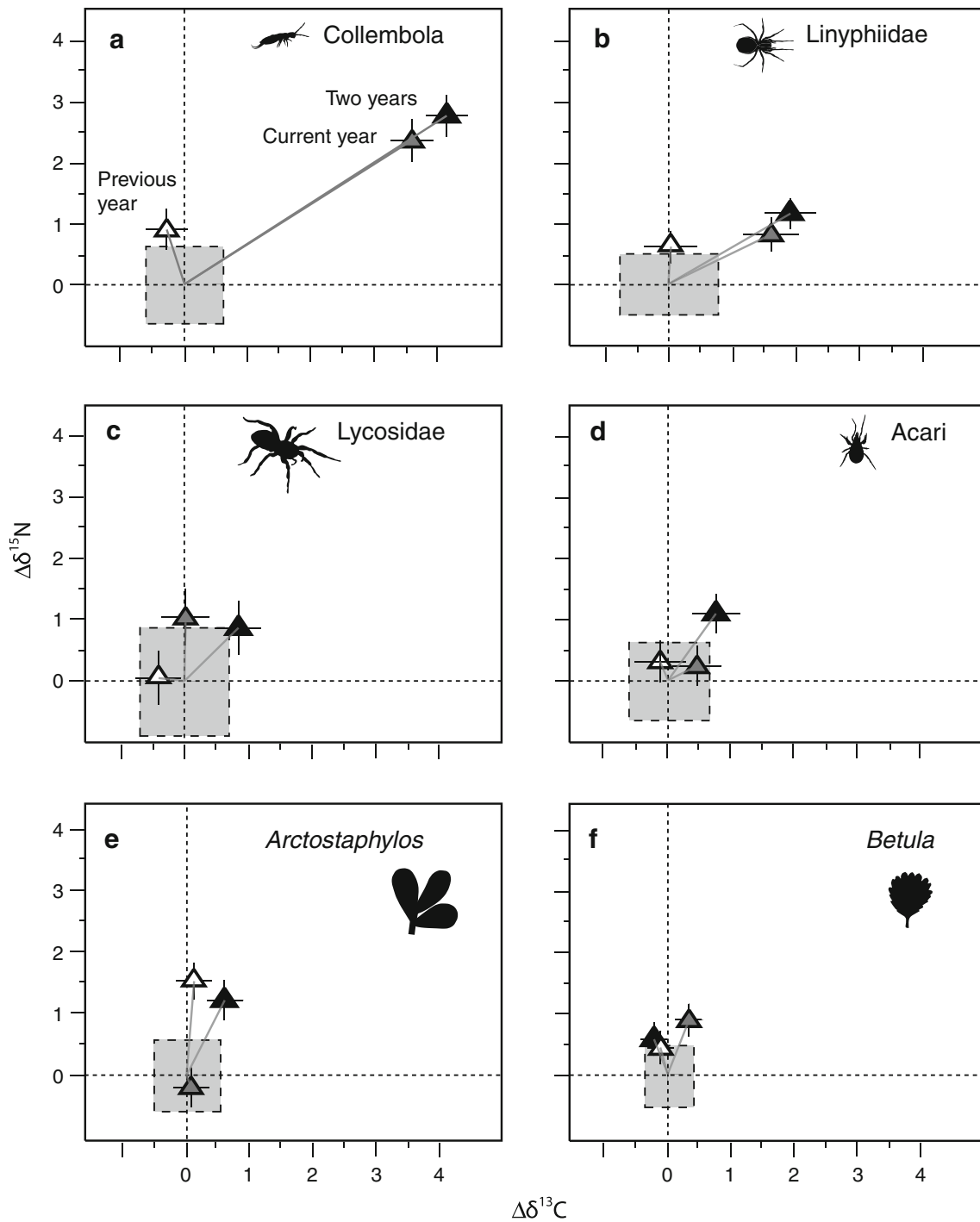


Fig. 2 Difference (mean \pm SE of the test statistic) between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of **a** Collembola **b** Linyphiidae, **c** Lycosidae, **d** Acari, and **e**, **f** the two dominant plants in control and midge-addition plots in 2009. The control value is represented by the origin. The critical area circumscribes the minimal difference required from the control $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to be significantly different at an α -level of 0.05

$\Delta\delta^{13}\text{C}$

(a priori contrast). *Current year* represents the “pulse-09” treatment; while *previous year* is the “pulse-08” treatment and *two years* is the “press” treatment. Significant differences between the midge-addition and control treatments are present in some treatments for all taxa shown, and Collembola isotopic values respond most strongly to midge addition

17–23 %, linyphiids 11–13 %, lycosids: 21–27 %) (Table 1). Overall, the posterior probability distribution of midges in consumer diets was higher when indirect

consumption was included (Table 1). Full Bayesian mixing model results (the proportion of each diet item) for each consumer are available in Appendix E in ESM.

Table 1 Mean (\pm credibility intervals) proportion of midges in the diet of a common detritivore (Collembola), mites (Acari) and two predators (linyphiid and lycosid) estimated using a Bayesian mixing model (SIAR) based on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of their bodies and diet sources

| Method | Treatment | Proportion of aquatic isotopes in diet | | | | | | | |
|-----------------------|---------------------------------|--|------------------|-------|------------------|-----------|------------------|-------------|------------------|
| | | Collembola | | Acari | | Lycosidae | | Linyphiidae | |
| | | Mean | 2.5–97.5 % CI | Mean | 2.5–97.5 % CI | Mean | 2.5–97.5 % CI | Mean | 2.5–97.5 % CI |
| “Direct and indirect” | Control | 0.06 | 0.01–0.13 | 0.17 | 0.11–0.24 | 0.21 | 0.13–0.28 | 0.11 | 0.04–0.17 |
| | Pulse 08 | 0.04 | 0.00–0.11 | 0.16 | 0.07–0.25 | 0.20 | 0.08–0.30 | 0.09 | 0.01–0.18 |
| | Pulse 09 | 0.35* | 0.27–0.42 | 0.22 | 0.14–0.30 | 0.19 | 0.10–0.28 | 0.24* | 0.14–0.33 |
| | Press | 0.38* | 0.30–0.45 | 0.23 | 0.12–0.32 | 0.27 | 0.19–0.35 | 0.25* | 0.15–0.36 |
| “Direct only” | Control | 0.03 | 0.00–0.08 | 0.04 | 0.01–0.08 | 0.11 | 0.05–0.18 | 0.01 | 0.00–0.03 |
| | Pulse 08 | 0.03 | 0.00–0.07 | 0.05 | 0.00–0.12 | 0.10 | 0.01–0.22 | 0.02 | 0.00–0.07 |
| | Pulse 09 | 0.18* | 0.06–0.28 | 0.03 | 0.00–0.10 | 0.06 | 0.00–0.16 | 0.04 | 0.00–0.13 |
| | Press | 0.20* | 0.07–0.31 | 0.03 | 0.00–0.10 | 0.07 | 0.01–0.19 | 0.04 | 0.00–0.14 |
| Indirect estimate | Cross-treatment mean difference | 0.10 | | 0.16 | | 0.13 | | 0.15 | |

Midge diet proportions in midge-addition treatments that have non-overlapping 97.5 % credibility intervals with controls are denoted with an asterisk. In the bottom row, the indirect diet contribution of midges is estimated by subtracting the mean “direct and indirect” proportion from the mean “direct” proportion for each taxa (all treatments combined)

Discussion

Our experimental additions of aquatic resources to a terrestrial food web show that midges are readily incorporated into arthropod decomposers (Collembola) and small spiders (Linyphiidae). After 2 years, aquatic isotopes are also apparent in larger spiders, mites and plants. This pattern of response suggests that the food web in this low-productivity heathland is able to quickly assimilate allochthonous resource pulses. However, the residence time of aquatic resources in terrestrial arthropods is limited. In the year following a resource addition (pulse-08 treatment), enriched C and N from aquatic sources are quickly immobilized or diluted with larger soil N pools.

Although the entire terrestrial food web was enriched with midge-derived ^{13}C and ^{15}N , detritivore isotope values responded more dramatically than predators or plants. The dominant arthropod detritivore in this system, Collembola, feed on decaying plants and animals, likely ingesting both detritus as well as microbes (Hopkin 1997). Collembola assimilated midge nutrients within the first year of midge addition (this effect was even detectable at very low sample size in 2008), and the isotopic response was consistent in the 2-year addition treatment. Among all measured members of the community, Collembola isotopic values changed most dramatically, suggesting they accessed midge resources most directly. Isotopic enrichment of Collembola in this experimental context strongly implies that these decomposers incorporated dead midges, and this effect propagated to predators.

Among predatory arthropods, linyphiid spiders were the only group whose isotopic values were enriched in the same year as midge addition, mirroring the response of Collembola. Linyphiids are small (0.5 mg dry weight), relatively sedentary sheet-web building spiders that live in plant litter and are therefore most likely to consume Collembola. Although they did not consume any dead midges directly, the proportion of midges in the linyphiid diet more than doubled (~ 10 to 25 %) in treatments that received midges in 2009 (pulse-09, press) compared to those that did not (pulse-08, control), strongly suggesting a trophic link to dead midges via Collembola.

In contrast to linyphiid spiders, the change in other predator isotope values was lower in magnitude and only detected after 2 years of midge addition. This delayed and dampened response suggests lycosids and mites are not trophically linked to Collembola as closely as linyphiids. Nevertheless, their enrichment demonstrates that midge nutrients that enter the detrital resource pool are subsequently incorporated into all abundant arthropods in the terrestrial food web. The delayed and apparently small effect of aquatic subsidies on lycosids may underestimate the importance of the subsidy because these predators do not feed on dead and dry midges (Nyffeler et al. 1994). However, adult aquatic insects usually fly to terrestrial systems and thus arrive as living biomass. Predators could benefit from live prey subsidies both directly by consuming midges and indirectly through increases in other arthropods. When spiders were sampled from Mývatn, a high-density midge lake, $\delta^{13}\text{C}$ values were 10 ‰ higher than spiders sampled at

Helluvaðstjörn, a low-density midge lake (Gratton et al. 2008), in contrast to a shift of 1–2 ‰ between midge-addition and control treatments for spiders in this experiment. Spiders near streams have also been shown to be isotopically enriched by aquatic insects, deriving 40–60 % of their C from aquatic sources near streams, but only 1 % 20 m from the stream (Collier et al. 2002; Briers et al. 2005). Isotopic enrichment highlights the pathways that aquatic insects can enter terrestrial food webs. Although direct consumption by predators is often implicitly assumed in isotope studies (Collier et al. 2002; Paetzold et al. 2006), our experiment isolated the detrital route and showed that terrestrial predators can also incorporate aquatic resources indirectly. Spiders do not feed on dry dead midges (Nyffeler et al. 1994) and diet inferences from mixing models confirmed that predators do not directly consume midges, though all arthropods incorporated midge-derived nutrients. Among analyzed taxa in the direct mixing model, only Collembola consumed midges. Including indirect midge incorporation dramatically increased aquatic isotope incorporation for all arthropods, thus highlighting the importance of indirect (detrital) pathways for aquatic-to-terrestrial subsidies.

The movement of animals in the heathland could also change the magnitude of the observed responses. Large hunting spiders like lycosids may be integrating prey resources across a larger area than our 1-m² experimental plots and may therefore have been less enriched than more sedentary taxa. Smaller spiders with more sedentary hunting modes, such as the sheet-web building linyphiids, were more enriched in midge-addition plots than larger more mobile lycosids, possibly because they were more likely to have fed exclusively in the plot where they were collected. Diptera larvae are perhaps the most sedentary arthropods in the food web and, along with their natural enemies, parasitoid wasps, appear to be the most highly enriched.

Although many taxa were isotopically enriched after one and especially two consecutive years of midge addition, isotopic values returned to background levels in the year following the termination of midge addition. The lack of carryover effects was surprising because the densities of several taxa (e.g., Collembola, mites) were still elevated in the year following a midge pulse (Hoekman et al. 2011). Given the short life span of these organisms, the discrepancy in density versus isotope carryover effects suggests that, even after midge C and N have left the arthropods, some nutrient effects persist for at least another year. The process by which this occurs is not known, but may be mediated by the plant community, which has more long-term responses to midge addition (Hoekman et al., unpublished data) and can in turn influence arthropod assemblages (Schaffers et al. 2008).

Our experiment demonstrated that some of the dominant plants in these heathlands can also incorporate midge-derived ¹⁵N into their tissues. Midges were likely initially broken down by microbes and entered the detrital food web, as evidenced by increased densities (Hoekman et al. 2011) and isotopically enriched arthropod detritivores. Through this process, some midge-derived nutrients entered the soil and were taken up by plants (DeAngelis 1992). Because of this detritus-processing step, we expected delayed responses of plants as was observed with *Arctostaphylos* which was only enriched in ¹⁵N in plots where midges were added the previous year. *Betula*, however, appeared to incorporate ¹⁵N from midge additions more quickly, showing a response even in the first year. Similarly, in a study conducted at the same latitude, N concentrations in leaf tissue increased within 1 year of experimental nutrient addition in tundra sedges (Shaver and Chapin 1995). The single-year response of *Arctostaphylos* and *Betula* differed, but both species incorporated midge-derived N after 2 years of midge addition, though there was no apparent cumulative effect of midge addition on plant isotope values.

Our study helps to inform our understanding of how resource pulses can cross ecosystem boundaries and link adjacent systems (Yang et al. 2010). These subsidies can arrive in many forms, including guano, salmon carcasses, sea wrack, cicadas, and aquatic insects, and can have large effects on community structure in recipient systems (Polis and Hurd 1995; Yang 2006; Hocking et al. 2009; Spiller et al. 2010; Dreyer et al. 2012). Because resource pulses are of high magnitude and short duration, predator satiation is common and pulsed resources often enter food webs via detrital pathways. While detrital resources may differ between systems, the methods of acquisition and assimilation via detrital pathways may be similar, including indirect utilization by predators. We found that arthropod detritivores were an important vector for aquatic resources entering terrestrial food webs. Although the effects of resource pulses in some ecosystems can be extraordinary, recipient ecosystems differ in their ability to retain allochthonous nutrients. We found midge-derived nutrients were short-lived in arthropod biomass, but they may be stored for longer periods in plant or soil resource pools. More long-term studies of pulsed cross-ecosystem subsidies are needed to understand storage mechanisms and persistent effects of resource pulses (Yang et al. 2008).

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