


Trophic transfer from aquatic to terrestrial ecosystems: a test of the biogeochemical niche hypothesis

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Abstract. Matter and energy flow across ecosystem boundaries. Transfers from terrestrial to aquatic ecosystems are frequent and have been widely studied, but the flow of matter from aquatic to terrestrial ecosystems is less known. Large numbers of midges emerge from some lakes in northern Iceland and fly to land. These lakes differ in their levels of eutrophication due to different intensities of geothermal warming and nutrient inputs. In the context of this material transfer from an aquatic to a terrestrial ecosystem, we investigated the relationships between the deposition of midges and the elemental composition and stoichiometry of organisms in low-productivity terrestrial ecosystems. We analyzed several terrestrial food webs in northeastern Iceland with similar food web compositions of terrestrial arthropods but different inputs of midges and analyzed the stoichiometric composition of the different trophic groups. Elemental composition differed among trophic groups and taxa much more than within each trophic group or taxa across the midge deposition gradient. Specifically, the change in N concentration was significant in plants (up to 70% increase in the site with maximum input) but not in predators, which had a more homeostatic elemental composition. These results thus show (1) a significant movement of matter and nutrients from an aquatic to a terrestrial habitat via the emergence of aquatic insects and the deposition of insect carcasses, (2) a larger impact on the elemental composition of plants than arthropods, and (3) support for the biogeochemical niche hypothesis, which predicts that different species should have a specific elemental composition, stoichiometry, and allocation as a consequence of their particular metabolism, physiology, and structure.

Key words: aquatic–terrestrial transfer; biogeochemical niche; food web; N:P; nitrogen; phosphorus; stoichiometry.

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INTRODUCTION

Aquatic and terrestrial ecosystems can be linked by the movement of matter and nutrients across habitat boundaries via the emergence of aquatic insects. Emergent aquatic insects alter trophic relationships and ecosystem productivity by the input of nutrients via insect carcasses (Gratton et al. 2008, Hoekman et al. 2011, 2012,

Bartrons et al. 2015). These aquatic subsidies of various bio-elements change nutrient availability in the terrestrial system (Gratton et al. 2017) and can thus trigger a change in elemental composition throughout the food web. This mechanism can be especially important in young terrestrial ecosystems with developing soils lacking critical nutrients. Terrestrial ecosystems in northern Iceland have low capacities to sustain biological

production because they contain poorly developed soils and experience harsh climatic conditions. Nevertheless, some lakes—such as Lake Mývatn—in the same area have notable production capacities due to geothermal inputs that provide warmth and nutrients (Einarsson et al. 2004). The aquatic subsidies from these productive lakes to the unproductive terrestrial landscape can be very large (Dreyer et al. 2012, 2015) and can have a fertilization effect in the surrounding area (Gratton et al. 2017). The input of midges from lakes to surrounding terrestrial ecosystems may moreover provide an opportune scenario to test hypotheses of ecological stoichiometry, such as the biogeochemical niche hypothesis (Peñuelas et al. 2008, 2010).

The biogeochemical niche hypothesis predicts that each species occupies a particular region of the multivariate space generated by the concentrations and ratios of macro- and micronutrients of its individuals (Peñuelas et al. 2008, 2010). This holistic view assumes that different species have a differential proportional use of elements in response to long-term evolutionary adaptation and as a result of their flexibility to respond to environmental changes. The species' optimal elemental concentrations and patterns of elemental allocation to different functions and organs are the consequences of not only adaptation to maximize species fitness in determined abiotic and biotic circumstances—that is, the consequence of long-term genetic adaptation—but also of the short-term capacity to respond, under certain limits, to lifelong environmental shifts and competitive circumstances (Peñuelas et al. 2008, 2010). Recent studies of the main forest species of Europe have shown that differences in foliar elemental composition among species can be explained by their phylogenetic distances, current climatic differences within their distributions, niche speciation in sympatric species, and recent anthropogenic impacts such as N deposition (Sardans and Peñuelas 2014, Sardans et al. 2015, 2016). In consumer–resource relationships, consumers' chemical composition is relatively homeostatic regardless of the chemical composition of its food (Sternner et al. 2002). However, nutrient content can still vary among consumer functional feeding and taxonomic groups, and size classes (Fagan et al. 2002, Cross et al. 2003, Fagan and Denno 2004), in addition to consumer

growth rate (Elser et al. 2000a) and relative allocation of structural biomolecules (Elser et al. 1996). For example, each taxa will be susceptible to reductions in growth or fitness whether food resources are low in N or P relative to body demand (e.g., Sternner et al. 1993). These findings illustrate the suitability and sensitivity of this concept for detecting the recent acclimation of species and populations to novel environmental circumstances, such as those imposed by human activity, but also long-term evolutionary adaptation to particular environmental conditions, such as nutrient inputs via aquatic deposition.

Our objectives are to: (1) investigate the importance of midge inputs to the elemental compositions of each taxon within each trophic group (plants, herbivores, predators, and detritivores), and (2) test whether each trophic group within a food web and each taxon within a trophic group has a specific biogeochemical niche. We conducted a study in northeastern Iceland where terrestrial ecosystems have similar species-specific food webs but differ in inputs from emergent aquatic insects due to the distance of these terrestrial systems from lakes and the productivity of each lake (Gratton et al. 2008, Dreyer et al. 2012). These emergent aquatic insects naturally fertilize the nutrient-poor terrestrial ecosystem, which is mostly nitrogen limited (Gratton et al. 2017), and set up ideal conditions to test the biogeochemical niche hypothesis at the whole food web level. We hypothesize that plants will show stronger stoichiometric differences among sites than arthropods, which we expect will be more similar to each other due to consumers' higher homeostatic capacity (Fagan et al. 2002, Cross et al. 2003, Fagan and Denno 2004).

MATERIALS AND METHODS

Study sites

We selected four terrestrial study sites in northeastern Iceland (~65°4' N, 17°0' W) with similar food web compositions of terrestrial arthropods but different inputs of aquatic insects (midges) (Gratton et al. 2008, Dreyer et al. 2012). We focused on the terrestrial food webs around four lakes spanning a gradient from extraordinarily high (Lake Mývatn), high (Lake Miklavatn), medium (Lake Botnsvatn) to low (Lake Helluvaðstjörn) densities of midge larvae, based

on Gratton et al. (2008). For example, midge deposition on land within 150 m of the shore was approximately 1000-fold higher at Lake Mývatn than Lake Helluvaðstjörn (Dreyer et al. 2012).

Primary and secondary productions in terrestrial ecosystems from this region just south of the Arctic Circle are limited by factors like soil pH, temperature (mean annual temperature of 14.3°C), a short growing season, and a short time when the mean temperature is above freezing (global radiation of 79 kcal cm²/yr) (Jónasson 1979).

Sample collection

The terrestrial arthropod community was sampled during the growing season (June/July) in 2006, 2008, 2010, 2011, and 2012. We sampled a total of eight transects in four areas around Lake Mývatn due to the size and spatial heterogeneity of insect emergence (two transects in Kálfaströnd [KAL], two transects in Skútustaðir [BTL], two transects in Haganes [HAG], and two transects in Vindbelgur [VIN]), and a total of two transects per lake at Lake Miklavatn (MIK), Lake Botnsvatn (BOT), and Lake Helluvaðstjörn (HEL). We sampled the plant and arthropod community at 5, 150, and 500 m from the shore along each transect. The sampling sites were dominated by heathland plant species.

For the analysis of elemental composition, we collected the shrubs *Betula nana* (L.), *Arctostaphylos uva-ursi* (L.), *Vaccinium uliginosum* (L.), *Empetrum nigrum* (L.), *Salix lanata* (L.), and *Salix phylicifolia* (L.); the grasses *Poa* spp. and *Deschampsia* spp.; the forbs *Galium verum* (L.) and *Achillea millefolium* (L.) and *Sphagnum* spp. bryophytes. Entire plants were collected, dried, and frozen until analysis.

Arthropods were collected by vacuum sampling with a modified leaf blower (SH 85 Shredder Vac/Blower, Stihl Incorporated, Virginia Beach, Virginia, USA). The vacuum sampler was pressed over the ground at each site to remove arthropods from the vegetation and leaf litter. The contents of each vacuum bag were emptied into white plastic trays in the field, and the arthropods were removed using ultra-clean plastic forceps and/or aspirators. If there were not enough of certain taxa, another sample was

taken. The same sampling equipment and methods were used in all locations. The dominant arthropod functional groups in the terrestrial food web are predators including: Lycosidae (wolf spiders, *Pardosa sphagnicola* [Dahl] and *Pardosa palustris* [Linnaeus, 1758]), Linyphiidae (sheet-web spiders, *Erigone arctica* [White, 1852]), Opiliones (harvestmen, *Mitopus morio* [Fabricius, 1779]), Carabidae (ground beetles), and Staphilinidae (rove beetles); detritivores including: Acari (mites, Trombididae) and Collembola (springtails); and herbivores including: Coccoidea (scale insects, *Arctorthezia cataphracta* [Olafsen 1772]) and Delphacidae (planthoppers, *Javasella pellucid* [F.]). In this system, arthropod community composition changes in response to midge addition (Hoekman et al. 2011) and some taxa are only prevalent in the presence of midge inputs. For the current study, we have focused our analysis on generalist species that are present at most sites. Larval and adult chironomid midges (*Tanytarsus* sp. and *Chironomus* sp.) were collected from each lake in 2008, 2010, and 2011. Larval midges were collected with emergence traps (described in Dreyer et al. 2015). Adult midges were collected with sweep nets (described in Hoekman et al. 2011).

The midge deposition estimated by terrestrial sampling (infall) along each transect and at each sample distance from the lake was collected using passive, lethal, aerial-infall traps set on stakes about 1 m high at 5, 150, and 500 m from the shore of each lake for estimating relative midge abundance on the shore, following Dreyer et al. (2012).

Analysis of elemental composition

Arthropod samples were freeze-dried, ground to a fine powder, and analyzed for elemental composition. “Large” organisms (e.g., wolf spiders) were ground individually, and a subsample of the resulting powder was used. “Small” organisms (e.g., Collembola) were pooled by species if a single individual did not result in enough material to compose a sample. Carbon (C) and nitrogen (N) concentrations were determined using a DELTA plus Advantage configured with a CONFLO III using a Carlo Erba NC2100 Elemental Analyzer (EA) at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, USA).

The concentrations of phosphorus (P), iron (Fe), titanium (Ti), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), zinc (Zn), copper (Cu), molybdenum (Mo), cadmium (Cd), antimony (Sb), lead (Pb), and sulfur (S) were determined by extraction by acid digestion in a MARSXpress microwave reaction system (CEM Corp., Matthews, North Carolina, USA) under high pressure and temperature. Briefly, 250 mg of dry sample powder was added to 5 mL of nitric acid and 2 mL of H₂O₂ in a Teflon tube. The digested material was transferred to 50-mL flasks and resuspended in Milli-Q water to a final volume of 50 mL. The elemental concentrations of each sample were determined by optic emission spectrometry with inductively coupled plasma (7500 ce ICP-MS; Agilent Technologies Inc., Waldbronn, Germany).

Data analysis

Linear mixed models were used to test the effects of midge infall on the scores of the first two axes of a principal component analysis (PCA), with C, N, P, Fe, Ti, Cr, Mn, Co, Ni, Zn, Cu, Mo, Cd, Sb, Pb, and S concentrations as variables (as a proxy of overall elemental composition), and on various elemental concentrations and concentration ratios at the levels of food webs, trophic groups (plants, herbivores, predators, and detritivores), and species. We used linear mixed models with midge input and trophic group as fixed factors and site as random factor. We also used linear mixed models with midge input and species or taxa as fixed factors and site, year, and distance as random factors to analyze the impact of midge input on the elemental compositions of the plant and predator communities (the corresponding univariate analyses in herbivores and detritivores were not done because they have a very low “n” in several sites and in general). These models identified possible impacts of midge input on the elemental compositions of overall plant and predator communities and the possible interactions of midge input and plant or predator taxa. Finally, we used linear mixed models with midge input as a fixed factor and site as a random factor to analyze the impact of midge input on the elemental composition of some of the most widespread plant (Poaceae: *B. nana* and *G. verum*) and predator species (Lycosidae: *P. sphagnicola* and *P. palustris*;

Linyphiidae: *E. arctica*; and Opiliones: *M. morio*) across the midge-input gradient. These three plant species (*A. uva-ursi*, *B. nana*, and *E. nigrum*) cohabited three of the sites (inland at different distances from Lakes Mývatn, Helluvaðstjörn, and Botnsvatn), and these three predator taxa (Linyphiidae, Lycosidae, and Opiliones) also cohabited three of the sites (Lakes Mývatn, Helluvaðstjörn, and Miklavatn), so we analyzed the changes in their overall elemental compositions at the sites and tested whether the elemental differences among the species were maintained among the sites. We chose the best model for each dependent variable using the Akaike information criterion. The MuMIn R package (Barton 2015) was used in the linear mixed models to estimate the percentage of variance explained by the models. The models were run with the “lme” function and the “mgcv” package (Wood 2011). We drew partial residual plots of the variability of N concentrations explained by midge infall (g·m⁻²·d⁻¹) across the entire food web, across plant species and across predator taxa using the visreg R package (Breheny and Burchett 2016). These partial residual plots evaluated the effect of each variable on a full model.

We also performed multivariate statistical analyses using general discriminant analysis (GDA) to determine the differences of elemental composition (depending on sites with different midge inputs) among and within the various trophic groups and plant and predator taxa. The GDAs and PCA were performed using Statistica 6.0 (StatSoft, Inc., Tulsa, Oklahoma, USA). We tested the biogeochemical niche hypothesis using a GDA, with the concentrations of 15 elements (C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Mo, Cd, and Pb) grouped by trophic group (plants, herbivores, predators, and detritivores) and site (KAL, BTL, VIN, and HAG at Lake Mývatn and Lakes Helluvaðstjörn (HEL), Miklavatn (MIK), and Botnsvatn [BOT]) at seven intensities of midge input (5.61 ± 1.03 (KAL), 2.17 ± 0.55 (BTL), 3.38 ± 1.33 (VIN), 2.55 ± 0.60 (HAG), 0.044 ± 0.005 (HEL), 0.562 ± 0.018 (MIK), and 0.163 ± 0.016 (BOT) g·m⁻²·d⁻¹, respectively) as categorical dependent variables.

All statistical analyses were performed in R software version 3.3.2 (R Development Core Team 2017).

RESULTS

Influence of midge input on the elemental composition of entire food webs, trophic groups, and taxa

The intensity of midge infall (midge deposition estimated by terrestrial sampling) was significantly correlated with organismal N concentration (Table 1) and marginally ($P < 0.1$) correlated with organismal P concentration but was not

Table 1. Linear mixed models with the scores of the principal component analysis of the C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Pb, and Mo concentrations, and the main bio-elements and bio-elemental ratios of all arthropod and plant communities as dependent variables; midge input and trophic group as fixed independent factors; and year, site, and distance as random factors (Model structure: lme(Y variable ~midge infall *trophic group. random= ~|year/site/distance)).

Variable	R^2 fixed factor	R^2 fixed + random factors	F fixed factor	P fixed factor
PC1 axis scores	0.52	0.57	midges 1.64 trophic group 39.4	0.20 <0.0001
PC2 axis scores	0.24	0.25	midges 1.32 trophic group 27.6	0.25 <0.0001
[C]	0.32	0.34	midges 0.22 trophic group 31.2 midges:trophic group 10.5	0.64 <0.0001 <0.0001
[N]	0.85	0.87	midges 4.76 trophic group 152	0.031 <0.0001
[P]	0.63	0.84	midges 3.26 trophic group 6.23 midges:trophic group 3.47	0.07 0.0004 0.017
C:N	0.52	0.54	midges 0.0019 trophic group 63.8 midges:trophic group 4.0	0.96 <0.0001 0.0083
C:P	0.48	0.51	midges 0.0077 trophic group 48.6 midges:trophic group 4.72	0.93 <0.0001 0.0032
N:P	0.12	0.32	midges 1.39 trophic group 12.1	0.24 <0.0001
[Fe]	0.075	0.13	midges 2.52 trophic group 5.08	0.11 0.002
[S]	0.13	0.13	midges 0.34 trophic group 13.0	0.56 <0.0001

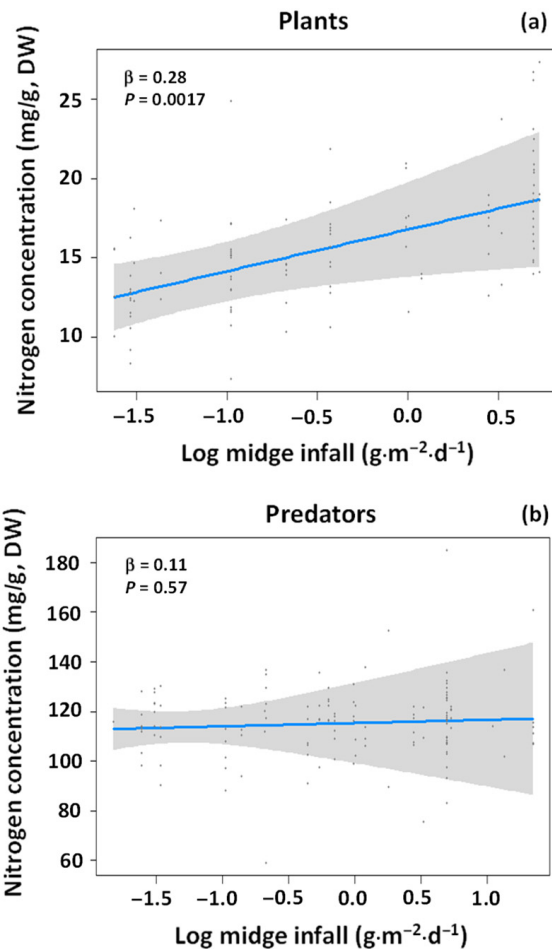


Fig. 1. Partial residual plot of the variability of N concentrations in plants (a) and predators (b) explained by log midge infall. The linear model included midge infall, taxonomic group, site, and year of sampling. Shaded areas indicate confidence bands (0.95).

correlated with the scores of the PC1 axis (for the PCA of organismic C, N, P, S, Fe, Ti, Mn, Co, Ni, Cu, Zn, Mo, Cd, Pb, and Sb concentrations). PC1 was mainly loaded by N, P, Zn, Cd, and Mo and PC2 by N, P, Co, Fe, Ti, and Mn. In the univariate analyses, all studied variables differed significantly among trophic groups (Table 1). Midge stoichiometric composition (i.e., C/N, C/P, and N/P) varied among lakes (MANOVA, $P = 0.02$). However, differences of these ratios among lakes were small (e.g., C/N, C/P, and N/P differences among Mývatn and Helluvaðstjörn were 0.3, 0.0, and 6.1, respectively), and in our mixed

Table 2. Linear mixed models with the scores of the principal component analysis of the C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Pb, and Mo concentrations, and the main bio-elements and bio-elemental ratios of all plant communities as dependent variables; midge input and plant taxon as fixed independent factors; and year, site, and distance as random factors (Model structure: lme(Y variable ~midge infall *taxon, random=~|year/site/location)).

Variable	R ² fixed factor	R ² fixed + random factors	F fixed factor	P fixed factor
PC1 axis scores	0.67	0.74	midges 0.30 taxon 11.3 midges:taxon 2.50	0.58 <0.0001 0.016
PC2 axis scores	0.62	0.64	midges 21.58 taxon 17.4	0.21 <0.0001
[C]	0.71	0.78	midges 0.62 taxon 23.9	0.43 <0.0001
[N]	0.70	0.81	midges 5.22 taxon 36.4	0.025 <0.0001
[P]	0.72	0.80	midges 5.26 taxon 35.3	0.025 <0.0001
C:N	0.77	0.83	midges 1.37 taxon 44.7	0.24 <0.0001
C:P	0.80	0.85	midges 0.19 taxon 32.5 midges:taxon 2.39	0.66 <0.0001 0.021
N:P	0.28	0.29	midges 0.11 taxon 4.41	0.74 <0.0001
[Fe]	0.67	0.69	midges 0.58 taxon 23.1	0.58 <0.0001
[S]	0.71	0.77	midges 1.45 taxon 23.2 midges:taxon 8.38	0.23 <0.0001 <0.0001

linear models, the results were unaffected by the inclusion of transect distance as a random factor. Thus, the distance from lake did not have a significant effect on the shifts in elemental composition.

The intensity of midge infall was positively correlated with N (Fig. 1a; Table 2) and P concentrations (Table 2) of plants but not with N and P concentrations of predators (Fig. 1b; Table 3). It was also correlated with overall plant elemental composition (Table 2).

The analysis of the effects of midge input on the three most widespread plant species—*Betula nana*, *Galium verum*, and Poaceae—indicated that midge input had no effect on *G. verum* (Table 4) but was correlated with several shifts in elemental concentrations in *B. nana* and Poaceae,

including the scores of PC1 and PC2 (Appendix S1: Fig. S1; Table 4). For *B. nana*, PC1 was mainly loaded by N, P, S, Fe, and Ti concentrations and PC2 by Zn, Pb, Mn, and Ti concentrations (Appendix S1: Fig. S1a), whereas for Poaceae, PC1 was mainly loaded by Co, Cr, Fe, Ti, and Zn concentrations and PC2 by N, P, Cu, and Ni concentrations (Appendix S1: Fig. S1c).

In the analysis of the effects of midge input on the three most widespread predator taxa—Linyphiidae, Lycosidae, and Opiliones—each had different scores on the PC1 axis (for the PCA of C, N, P, Ca, Mg, Fe, Ti, Mn, Cu, Ni, Cd, Zn, and Sb concentrations); different C, N, P, and Ca concentrations; and different C:N, C:P, and N:P concentrations ratios under different intensities of midge infall (Table 3). Midge input had few effects on Lycosidae species, only affecting C concentrations (Table 5) but

Table 3. Linear mixed models with the scores of the principal component analysis of the C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Pb, and Mo concentrations, and the main bio-elements and bio-elemental ratios of all predator communities as dependent variables; midge input and predator taxon as fixed independent factors; and year, site, and distance as a random factor (Model structure: lme(Y variable ~midge infall *taxon, random=~|year/site/distance)).

Variable	R ² fixed factor	R ² fixed + random factors	F fixed factor	P fixed factor
PC1 axis scores	0.19	0.33	midges 1.61 taxon 6.64	0.31 <0.0001
PC2 axis scores	0.010	0.10	midges 2.61 taxon 2.57	0.13 0.022
[C]	0.15	0.15	midges 9.0 taxon 2.6	0.033 0.029
[N]	0.25	0.28	midges 1.70 taxon 9.02	0.19 <0.0001
[P]	0.14	0.78	midges 1.82 taxon 14.7	0.18 <0.0001
C:N	0.43	0.50	midges 0.036 taxon 21.4	0.85 <0.0001
C:P	0.28	0.61	midges 2.39 taxon 16.6	0.13 <0.0001
N:P	0.18	0.53	midges 4.1 taxon 8.65	0.045 <0.0001
[Fe]	0.050	0.086	midges 1.39 taxon 1.33	0.24 0.26
[S]	0.10	0.13	midges 0.136 taxon 3.02	0.71 0.014

Table 4. Linear mixed models with the scores of the principal component analysis of the C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Pb, and Mo concentrations, and the main bio-elements and bio-elemental ratios of the three most widespread plant species as dependent variables; midge input as a fixed independent factor; and year, site, and distance as a random factors (Model structure: lme(Y variable ~midges input. random= ~|year/site/distance)).

Species	Variable	R^2 fixed factor	R^2 fixed + random factors	F fixed factor	P fixed factor
<i>Betula nana</i>	PC1 axis scores	0.20	0.93	5.89	0.041
	PC2 axis scores	0.0054	0.96	0.64	0.45
	[C]	0.0052	0.12	0.087	0.78
	[N]	0.34	0.87	10.3	0.018
	[P]	0.13	0.86	32.4	<0.0001
	C:N	0.30	0.92	8.22	0.028
	C:P	0.07	0.85	3.41	0.090
	N:P	0.039	0.66	0.19	0.68
	[S]	0.34	0.87	0.3	0.018
	[Fe]	0.020	0.98	0.29	0.61
<i>Galium verum</i>	PC1 axis scores	0.005	0.96	0.24	0.89
	PC2 axis scores	0.057	0.68	1.12	0.37
	[C]	0.0070	0.71	0.14	0.73
	[N]	0.011	0.78	3.21	0.17
	[P]	0.062	0.65	0.82	0.43
	C:N	0.12	0.95	1.79	0.28
	C:P	0.03	0.63	0.34	0.60
	N:P	0.012	0.29	0.16	0.72
	[S]	0.025	0.80	0.69	0.47
	[Fe]	0.005	0.91	0.034	0.86
Poaceae	PC1 axis scores	0.05	0.98	1.43	0.28
	PC2 axis scores	0.21	0.21	5.75	0.029
	[C]	0.0059	0.0060	0.132	0.72
	[N]	0.22	0.36	5.95	0.039
	[P]	0.31	0.31	9.76	0.0066
	C:N	0.20	0.76	7.53	0.036
	C:P	0.32	0.62	9.38	0.028
	N:P	0.0084	0.30	0.023	0.65
	[Fe]	0.19	0.99	3.21	0.11

was correlated with several shifts in elemental concentrations in Linyphiidae and Opiliones taxa, including N concentrations and C:P and N:P ratios (Table 5).

Biogeochemical niche

The same taxa at the various sites tended to be grouped and separated relative to the other trophic groups (Fig. 2; Appendix S1: Tables S1, S2). The squared Mahalanobis distances, however, did not differ significantly between the detritivores and the predators and herbivores for HAG, BTL, and KAL (all at Lake Mývatn) (Appendix S1: Table S1). This analysis again indicated that C, N, and P concentrations were the most determinant variables in the models explaining the overall differences in elemental

compositions among the taxonomic groups at the sites (Appendix S1: Table S2).

Each plant taxon was significantly separated from all other taxa in the GDA, independent of site, with C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Mo, Cd, and Pb concentrations as grouping factors (Appendix S1: Tables S3, S4). The GDA was driven primarily by differences in C, N, S, Co, Cu, and Zn concentrations, which significantly separated each plant taxon from all other taxa, independent of the site (Appendix S1: Tables S3, S4). Each predator taxon was significantly separated from all other taxa in the GDA, independent of site, with C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Mo, Cd, Sb, and Pb concentrations as grouping factors (Appendix S1: Tables S3, S4). Differences in C, N, P, Cu, and Zn

Table 5. Linear mixed models with the scores of the principal component analysis of the C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Pb, and Mo concentrations, and the main bio-elements and bio-elemental ratios of the three most widespread predator species as dependent variables, midge input as a fixed independent factor, and site as a random factor.

Species	Variable	Model structure: lme(Y variable ~midges, random= ~ year/site/distance)			
		R^2 of the model		Midge-input statistics in the model	
		R^2 fixed factor	R^2 (fixed + random) factors	F	P
Linyphiidae	PC1 axis scores	0.041	0.085	1.49	0.25
	PC2 axis scores	0.047	0.58	2.75	0.11
	[C]	0.036	0.10	1.42	0.24
	[N]	0.098	0.69	3.32	0.041
	[P]	0.0027	0.57	0.22	0.65
	C:N	0.0083	0.56	0.22	0.64
	C:P	0.10	0.65	5.15	0.042
	N:P	0.11	0.69	7.4	0.023
	[Mg]	0.018	0.2	0.69	0.42
	[Fe]	0.013	0.14	0.47	0.50
	[Ca]	0.024	0.024	0.84	0.40
Lycosidae (<i>Pardosa sphagnicola</i> and <i>P. palustris</i>)	PC1 axis scores	0.0083	0.48	0.43	0.51
	PC2 axis scores	0.065	0.32	2.64	0.11
	[C]	0.11	0.11	6.34	0.017
	[N]	0.012	0.17	0.78	0.40
	[P]	0.001	0.78	1.1	0.32
	C:N	0.006	0.19	0.56	0.54
	C:P	0.067	0.77	0.38	0.17
	N:P	0.0028	0.64	0.16	0.69
	[Mg]	0.0043	0.22	0.20	0.66
	[Fe]	0.0039	0.53	0.15	0.70
	[Ca]	0.31	0.36	1.28	0.27
Opiliones (<i>Mitopus morio</i>)	PC1 axis scores	0.0083	0.48	13.2	0.0025
	PC2 axis scores	0.038	0.21	0.90	0.36
	[C]	0.020	0.20	0.55	0.50
	[N]	0.023	0.094	0.58	0.46
	[P]	0.09	0.87	6.31	0.024
	C:N	0.015	0.81	0.38	0.55
	C:P	0.015	0.66	10.7	0.0052
	N:P	0.16	0.68	12.2	0.0033
	[Fe]	0.38	0.41	17.4	0.0008
	[Ca]	0.18	0.19	5.86	0.029
	[Mg]	0.29	0.30	11.2	0.0045

concentrations were the most important among each taxon (Appendix S1: Table S6).

Each plant taxon had a unique elemental composition, and all pairwise comparisons among species identified different overall elemental compositions (significantly different squared Mahalanobis distances), independent of the site (Fig. 3a, b; Appendix S1: Tables S7, S8). The two-first axes separated significantly the scores of different trophic groups (Fig. 3), and they both represented a 89.3% (plants) and 60% (predators) of the whole variance. S, Fe, Ti, and Pb

concentrations explained most of these differences (Appendix S1: Table S8).

The GDA with C, N, S, P, Ca, Fe, Ti, Mn, Ni, Cu, Zn, and Cd concentrations as grouping variables and the three species at the three sites ($3 \times 3 = 9$ groups) as categorical dependent variables indicated that the predator taxa had unique elemental compositions and that all pairwise comparisons between species identified different overall elemental compositions (significantly different squared Mahalanobis distances), independent of the site (Fig. 3c, d; Appendix S1: Table S9). S, Fe,

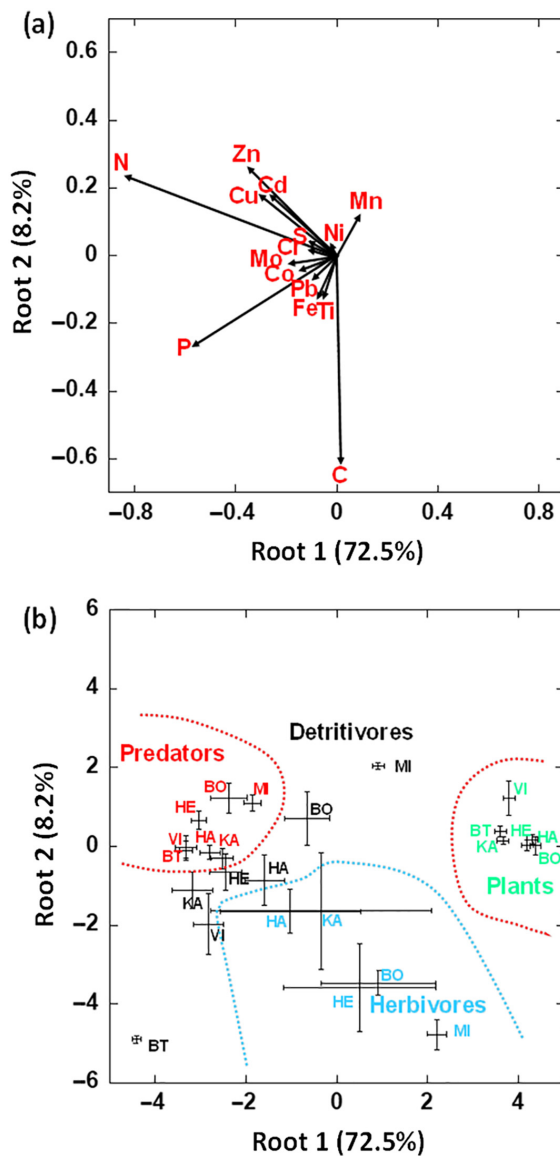


Fig. 2. The general discriminant analysis of the concentrations of 15 elements (C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Mo, Cd, and Pb) as grouping factors (a) and the trophic groups (plants, herbivores, predators, and detritivores) and the seven study sites (KAL, BTL, VIN, and HAG at Lake Mývatn and Lakes Helluadstjörn, Miklavatn, and Botnsvatn) (b), each with different intensities of midge infall (5.61 ± 1.03 , 2.17 ± 0.55 , 3.38 ± 1.33 , 2.55 ± 0.60 , 0.044 ± 0.005 , 0.562 ± 0.018 , and $0.163 \pm 0.016 \text{ g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, respectively) as categorical dependent variables.

Ti, and Pb concentrations explained most of these differences (Fig. 3c, d; Appendix S1: Table S10). Linyphiidae specimens had different overall elemental compositions, depending on the site, whereas Opiliones and Lycosidae specimens had the same overall elemental composition in two of the three studied sites (Fig. 3c, d; Appendix S1: Tables S9, S10).

DISCUSSION

Trophic transfer from aquatic to terrestrial ecosystems

Previous studies have demonstrated that emerging aquatic insects transfer matter and energy, increase arthropod biomass, and thus influence the trophic relationships of the surrounding terrestrial ecosystems (Gratton and Vander Zanden 2009, Hoekman et al. 2011, 2012). In addition, other studies have observed different elemental composition and stoichiometry among different trophic levels and/or taxon (Sardans et al. 2012). However, to our knowledge, the impacts of aquatic insects on overall elemental composition and stoichiometry of surrounding terrestrial food webs including plants have not been studied.

Midge input had a significant effect on the elemental composition of the food web overall but shifted elemental concentrations differently in each trophic group. Notably, midge input significantly increased the N concentration in plants up to 70% but did not significantly change the N concentration in predators. The results thus show a significant movement of matter and nutrients from an aquatic to a terrestrial habitat via the emergence of aquatic insects and the deposition of insect carcasses. This movement of aquatic insects alters trophic relationships and ecosystem productivity (Dreyer et al. 2012).

Plants have a modular structure that favors a more flexible body elemental composition response than arthropods that have more unitary and defined body form and general structure. These results are also consistent with most ecological stoichiometric studies showing a more homeostatic stoichiometry in arthropods than in plants (Sternner et al. 2002, Persson et al. 2010,

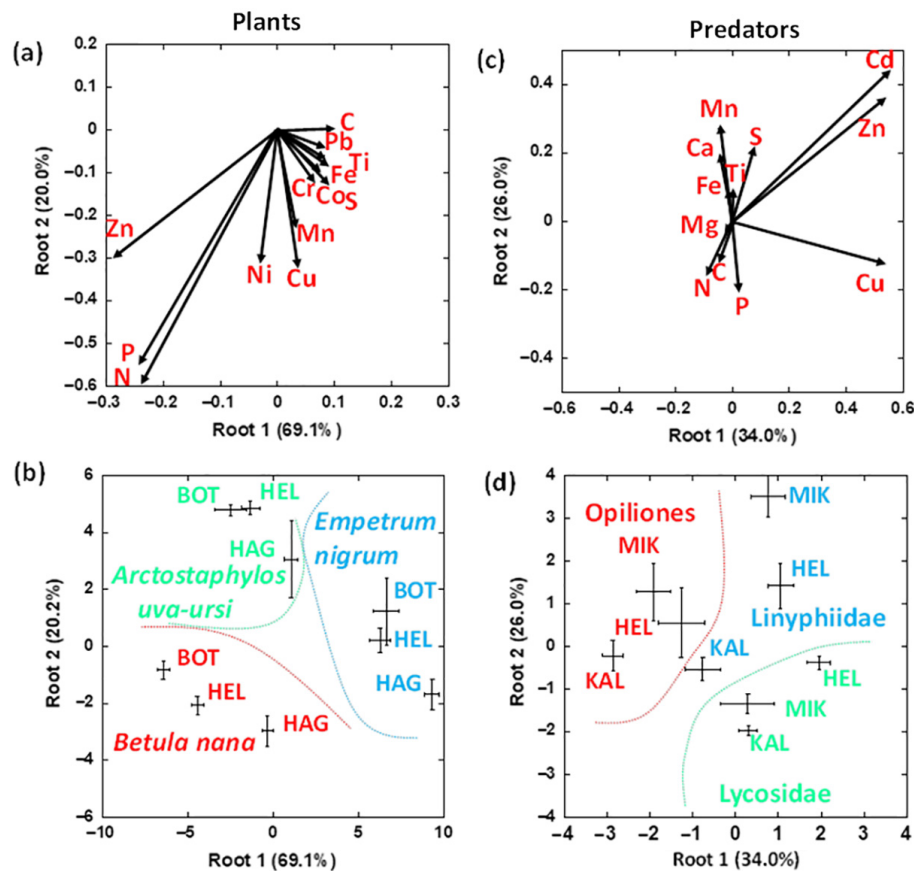


Fig. 3. Variable distributions in the two-first roots of the general discriminant analysis (a, c) and the corresponding score distributions (b, d). The concentrations of 15 elements (C, N, S, P, Fe, Ti, Cr, Mn, Co, Ni, Cu, Zn, Mo, Cd, and Pb) are the grouping factors for the three most widespread plant taxa (*Arctostaphylos uva-ursi*, *Betula nana*, and *Empetrum nigrum*) (a, b) and the three most widespread predator taxa (Opiliones, Lycosidae, and Linyphiidae) (c, d). The sites (categorical variables) are HAG at Lake Mývatn, and the lakes Helluvaðstjörn, HEL, and Botnsvatn, BOT, for the plants; and KAL at Lake Mývatn, and the lakes Helluvaðstjörn, HEL, Miklavatn, MIK, and Botnsvatn, BOT, for the predators).

Sardans et al. 2012). To reestablish differences in stoichiometric resource quality, consumers exert compensatory actions by altering their consumption rates to maintain the supply of required elements (compensatory feeding), but also and to a lesser extent, by shifting their body stoichiometry, or by avoiding habitats with low-quality resources (Jochum et al. 2017).

The great increase in nutrient concentrations in several taxa including most plants, together with the results of previous studies observing increases in biomass in these communities due to midge infall (Hoekman et al. 2011, Dreyer et al. 2012), demonstrates a notable increase in

nutrient contents in terrestrial biomass as a result of midge transfer from aquatic to terrestrial ecosystems. These results thus suggest that this transfer of midges increases soil fertility, enhances the processes of soil formation, and thus acts as plant growth catalyzer changing plant stoichiometry in these ecosystems, in their initial phases of development. Future studies may investigate the effects of elevated plant N on other trophic levels and potential consequences for trophic dynamics and community structure in these systems where climatic constraints of subarctic conditions slow these processes.

Biogeochemical niche

Trophic groups had specific elemental compositions and concentrations of several bio-elements, such as C, N, P, and S, and associated concentration ratios, such as C:N, C:P, and N:P. These similarities were maintained within trophic groups, despite the influence that midge input has on the elemental composition and concentration of particular taxa. Detritivores were the exception, because their elemental composition overlapped with those of the herbivores and predators. These results suggest that the elemental composition of detritivore bodies is less homeostatic, probably due to a wider variety of food sources in their diet (from midges to other arthropods and plant litter). Previously published studies on detritus-based food webs (Cross et al. 2003) also point to an evolutionary adaptation of detritivores to low nutrient food resources via lowered body nutrient content (Elser et al. 2000b, Fagan et al. 2002) and, in some detritivore taxa (e.g., *Tipula* spp., Diptera), to a specific gut flora or fauna that potentially aid in the assimilation of low nutrient resources (Klug and Kotarski 1980). Elemental compositions were similar for the Opiliones, which are partly detritivores, across the gradient of midge input. The biogeochemical niche hypothesis was thus generally supported, with specific elemental compositions among the trophic levels and with the maintenance of these differences irrespective of the site and midge input.

This study thus provides the first evidence that different trophic groups and arthropod taxa can have distinct elemental compositions among them irrespective of the habitat (nutrient fertilization in this case due to midge inputs of each lake and distance) but can also have minor but significant differences (within groups) dependent on the habitat. These results are consistent with previous studies testing biogeochemical niches in other scenarios, from European forests to common garden experiments (Sardans and Peñuelas 2014, Sardans et al. 2015, 2016, Urbina et al. 2015), detritus-based streams (Cross et al. 2003), salt marshes (Fagan et al. 2002, Fagan and Denno 2004), marine planktonic food webs (Moorthi et al. 2016), and tropical invertebrate communities (Jochum et al. 2017). They are also consistent with the trade-off equilibrium between homeostasis and the plasticity of species' elemental composition (Yu et al. 2010). The

homeostasis of elemental composition is due to the optimized metabolic and physiological functions and morphologies developed over long periods of time, resulting in each species reaching an optimal chemical composition associated with a single optimal function. In addition, different species (and different functional groups) have, to some degree, a flexible adaptive capacity to alter their elemental stoichiometries in response to changes in the composition of neighboring species and/or in environmental conditions (such as climatic gradients) (Fagan and Denno 2004, Sardans et al. 2011, Sardans and Peñuelas 2014, Jochum et al. 2017). This flexibility should be due to both a long-term adaptive acquired trait (genotype) and to mechanisms of genotypic expression (phenotype). Species thus exhibit a certain degree of stoichiometric flexibility, probably with a trade-off between adaptive capacity (flexibility) and stability (homeostasis) (Yu et al. 2010).

Final remarks and conclusions

The intensity of midge input affected the elemental composition of the food webs by affecting the N and P concentrations of the organisms, but with the changes being dependent on the trophic group, significant for plants but not for predators. The results suggest that the use of the different nutrients provided by the midges varies among the trophic groups and within each trophic group by different taxa.

The results were consistent with the biogeochemical niche hypothesis in the sense that each biological level (trophic and taxonomic groups) had unique elemental compositions based on its function and morphological structure, but with some level of flexibility of elemental composition depending on environmental circumstances.

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