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Ecological stoichiometry and nutrient partitioning in two insect herbivores responsible for large-scale forest disturbance in the Fennoscandian subarctic

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Abstract. 1. Outbreaks of herbivorous insects can have large impacts on regional soil carbon (C) storage and nutrient cycling. In northernmost Europe, population outbreaks of several geometrid moth species regularly cause large-scale defoliation in subarctic birch forests. An improved understanding is required of how leaf C and nutrients are processed after ingestion by herbivores and what this means for the quantity and quality of different materials produced (frass, bodies).

2. In this study, larvae of two geometrid species responsible for major outbreaks (*Epirrita autumnata* and *Operophtera brumata*) were raised on exclusive diets of *Betula pubescens* var. *czerepanovii* (N. I. Orlova) Hämet Ahti and two other abundant understorey species (*Betula nana*, *Vaccinium myrtillus*). The quantities of C, nitrogen (N) and phosphorus (P) ingested and allocated to frass, bodies and (in the case of C) respired were recorded.

3. Overall, 23%, 70% and 48% of ingested C, N and P were allocated to bodies, respectively, rather than frass and (in the case of C) respiration. *Operophtera brumata* consistently maintained more constant body stoichiometric ratios of C, N and P than did *E. autumnata*, across the wide variation in physico-chemical properties of plant diet supplied.

4. These observed differences and similarities on C and nutrient processing may improve researchers' ability to predict the amount and stoichiometry of frass and bodies generated after geometrid outbreaks.

Key words. Consumer-driven nutrient recycling, ecological stoichiometry, geometrid moth, homeostasis, stable isotope, subarctic birch forest.

Introduction

The key nutrients limiting plant growth in high-latitude forests are nitrogen (N) and, in some cases, phosphorus (P) (Vitousek & Howarth, 1991; Giesler *et al.*, 2004), but the effects of herbivores on ecosystem-level availability of these nutrients remain poorly understood (Hunter, 2001; Bardgett & Wardle, 2003; Hartley & Jones, 2008; Grüning *et al.*, 2017; Sitters *et al.*, 2017). Most research to date on the ecosystem effects of herbivores has focused on large mammals (Pastor *et al.*, 1988; Augustine & McNaughton, 1998; Olofsson *et al.*, 2004). By comparison with mammals, less is known about the role of insect herbivores, although available studies suggest that insect population outbreaks can exert major impacts on ecosystem structure and function (Volney & Fleming, 2000; Kaukonen *et al.*, 2013; Metcalfe *et al.*, 2013, 2016). One well-known example of insect herbivores which produce ecosystem-altering outbreaks is the geometrid moth species infesting mountain birch [*Betula pubescens var. czerepanovii* (N. I. Orlova) Hämet

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Ahti] forests across Fennoscandia at regular intervals (Haukioja *et al.*, 1988; Tanhuanpää *et al.*, 2002; Tenow *et al.*, 2007; Jepsen *et al.*, 2009). The geometrid species responsible for the largest outbreaks in Fennoscandia are the larvae of the autumnal moth (*Epirrita autumnata*) and winter moth (*Operophtera brumata*). The spatiotemporal patterns of moth outbreaks and defoliation (Ims *et al.*, 2004; Tenow *et al.*, 2007; Jepsen *et al.*, 2008) and observations of the end results of defoliation on vegetation and soils (Jepsen *et al.*, 2013; Kaukonen *et al.*, 2013; Saravesi *et al.*, 2015; Parker *et al.*, 2016; Kristensen *et al.*, 2018) have been studied. By comparison, there is limited information available on the intermediate steps and underlying mechanisms linking macro-scale observations of outbreaks to the longer-term consequences for ecosystem biogeochemistry. In part, this lack of data reflects the difficulty inherent in bridging the disparate disciplines of biochemistry, population biology and community ecology which is necessary to understand consumer-driven nutrient recycling (Hunter & Price, 1992; Hunter, 2001; Pomeroy, 2001).

A range of novel tools have emerged to study trophic linkages between primary producers and consumers, and their biogeochemical impacts, such as the use of ecological stoichiometry and stable isotope abundances. Isotopic enrichment of stable isotopes of C and N derived from food material during herbivore digestion provides important clues about diet and trophic relationships (Post, 2002), but the usefulness of the approach in community ecology is critically limited by the paucity of experimental studies tracing shifts in stable isotopes from source food material to different herbivore products (Gannes *et al.*, 1997; Ben-David & Schell, 2001; Caut *et al.*, 2008, 2009).

The quantity of ingested C, N and P diverted to different herbivore products is the end result of several steps (Waldbauer, 1968; Scriber & Slansky, 1981). First, ingestion rate clearly controls the absolute magnitude of plant matter removed and potentially available to the herbivore. Second, the proportion of ingested material which is digested and absorbed (approximate digestibility, or AD) controls how much of the resources ingested become available for growth and metabolism. Third, the proportion of ingested food converted to insect bodies (efficiency of conversion of ingested food, or ECI) determines most directly the allocation of resources among herbivore products. All these steps are interlinked and may vary substantially according to herbivore life strategy and plant chemical quality/defences. For example, ingestion rate often tends to increase with a decrease in the limiting nutrient due to compensatory feeding (Berner *et al.*, 2005), while plant material of low chemical quality or with high concentrations of defence compounds may suppress AD and/or ECI through various mechanisms (e.g. altered gut passage time, elevated respiratory rates; Berner *et al.*, 2005, Clissold *et al.*, 2009, Cresswell *et al.*, 1992, Raubenheimer & Simpson, 1999). The extent of homeostatic control over internal C, N, P ratio could prove useful in predicting the scale and spatial pattern of potential range shifts with climate change (Ward & Masters, 2007; Gonzalez *et al.*, 2010). If these predictions hold across a diversity of herbivore types and host plant species, they would potentially provide a framework to link plant chemical traits to herbivore-mediated nutrient fluxes, and anticipate differences in responses among herbivores to environmental changes, thereby facilitating improved integration of herbivore activity into global models (Throop *et al.*, 2004; Ostle *et al.*, 2009).

The aims of this manuscript were to describe partitioning of C, N and P by two widespread insect herbivore species in the Fennoscandian subarctic, and to quantify how the pattern of partitioning and the chemical composition of herbivore products were affected by the chemical content of the plant species in the herbivore diet. We quantified the pathways for C, N and P after defoliation by raising *E. autumnata* and *O. brumata* larvae on exclusive diets of *B. pubescens*, *B. nana* (dwarf birch) and *Vaccinium myrtillus* (bilberry), and then recording the pattern of ingested C, N and P partitioned to bodies, frass and (in the case of C) respiration. We ask how post-ingestion pathways for C, N and P vary among herbivore species and among plant diets. Further, for each herbivore × plant species combination, we assess possible stoichiometric controls over observed differences by quantifying AD, ECI and homeostasis, and provide values of post-ingestion isotopic enrichment of ^{13}C and ^{15}N for potential use in future studies on diet and trophic relationships within the study system.

Materials and methods

Study system

The study area was around Tromsø in northern Norway (69°38′56.6″N 18°57′17.1″E), whicih has an oceanic climate with mild and snow-rich winters and cool summers. The annual precipitation is ∼1000 mm and the mean temperature in January is −4.4 °C and, in July, 9.1 °C. The forest of the region is dominated by *B. pubescens* with understorey species like *B. nana*, *V. myrtillus*, northern bilberry (*Vaccinium uliginosum*) and black crowberry (*Empetrum nigrum* ssp. *hermaphroditum*).

Measurements

All samples for feeding material and larvae were collected in early June 2015 within 10 km of Tromsø. A total of 660 larvae in the second-third instar were picked from the canopies of *B. pubescens* individuals. In the field, *E. autumnata* tends to develop faster than *O. brumata* (Mjaaseth *et al.*, 2005) so it is possible that a relatively greater portion of sampled *E. autumnata* larvae were at the third instar than was the case for sampled *O. brumata*. Both moth species can be subject to parasitoids (Virtanen & Neuvonen, 1999), but the occurrence of parasitoids and prevalence of larval parasitism were not surveyed during sampling, although no parasitoids were observed emerging from larvae during the experiment. Ten larvae each of *E. autumnata* and *O. brumata* at second-third instar were dried at 60 °C for 48 h and weighed separately. Thus, the larvae were raised on a natural diet for one to two instars before inclusion in the experiment. For *E. autumnata*, 20 live larvae were placed in six boxes, each filled with fresh leaves from only one plant species: *B. pubescens*, *B. nana* or *V. myrtillus* (three plant species × six replicates = 18 boxes in total). For *O. brumata*, 20 live larvae were placed within five boxes, each filled with fresh leaves from only one plant species:

B. pubescens, *B. nana* or *V. myrtillus* (three plant species × five replicates = 15 boxes in total). The boxes containing the larvae were kept in an illuminated room with a constant temperature of 15 °C to ensure optimal growth. The leaves in the boxes were removed and weighed every fourth day and replaced with a known amount of fresh leaves. Fresh leaves were sampled from five *B. pubescens* trees and eight individuals of *B. nana* and *V. myrtillus* within 10 km of Tromsø. Frass was removed at every leaf change, dried at 60 °C for 48 h and then weighed. After 1 month, the pupae and unpupated larvae from each box were counted, dried at 60 °C for 48 h and weighed separately.

Calculations

Mean dry mass of individual second-instar larvae of each species was multiplied by 20 to estimate total dry larval body biomass per box at the start of the experiment. This initial dry larval body biomass per box was subtracted from the combined biomass of pupae and larvae bodies at the end of the experiment in each box, to estimate dry biomass accumulated in living herbivore bodies over the project duration. Larval survival was calculated as the proportion of initial larvae that either were alive at the end of the experiment or had successfully pupated per box. Observations of survival patterns among herbivore species in this experiment should be interpreted with caution because they could be affected by: (i) possible differences in median development stage of larvae selected per species (see 'Measurements' earlier); and (ii) differences in how effectively the different herbivore species can be raised in artificial mesocosms. Further, causes of mortality (larval parasitism etc) were not identified. Separate frass collections per box were pooled to calculate total frass dry mass generated over the entire experimental duration per box. Leaf samples for analysis were collected at the beginning of the experiment, so foliar chemistry does not reflect possible phenological shifts over the project duration. In addition, larvae and pupae from each box were pooled to derive total herbivore body samples per box. Foliage samples from each of the three plant species studied together with pooled frass, pupae and larvae samples from each of the six herbivore−plant combinations were subjected to chemical analyses to determine total C and N as well as their isotopic ratios with double determination of 2 mg ball-milled solid samples exposed to Dumas combustion (1020 °C) on an elemental analyser (CE 1110; Thermo Electron, Milan, Italy) coupled in continuous-flow mode to an isotope ratio mass spectrometer (Finnigan MAT Delta PLUS; Thermo Scientific, Bremen, Germany) and total P content [25 mg ball-milled leaf material digested in 25 ml sulphuric acid with selenium as catalyst (Kedrowski, 1983) followed by spectrophotometry with the molybdenum-blue method]. The chemistry of herbivore products and diet is summarised in Table 1. It was necessary to pool material collected from replicate boxes to obtain sufficient

Table 1. Mean chemical properties of plant leaves and products from herbivores raised exclusively on the leaves of the selected plant species.

	Epirrita autumnata			*Operophtera brumata*		
	Leaves	Bodies	Frass	Leaves	Bodies	Frass
Betula pubescens						
Carbon content (%)	49.3	43	40.7	51.0	51.3	25.7
Nitrogen content (%)	3.4	9.3	2.2	3.6	9.8	1.4
Phosphorus content (%)	0.35	0.47	0.28	0.35	0.45	0.22
$\delta^{13}C$	−30.2	−30.9	−31.0	−29.8	−30.9	−30.6
$\delta^{15}N$	−3.0	0.7	−0.6	−3.1	1.0	−1.9
C:N ratio	14.5	4.6	18.3	14.3	5.3	18.5
C:P ratio	142	91	148	145	114	119
N:P ratio	10	20	8	10	22	6
Betula nana						
Carbon content (%)	48.4	49.3	47.6	47.7	44.6	26.6
Nitrogen content (%)	2.9	9.0	2.3	3.1	8.7	1.4
Phosphorus content (%)	0.30	0.50	0.24	0.30	0.43	0.20
$\delta^{13}C$	−31.5	−30.1	−31.0	−31.3	−30.8	−31.3
$\delta^{15}N$	−1.5	0.1	−1.9	−0.7	1.1	−1.8
C:N ratio	16.8	5.5	20.6	15.3	5.1	19.2
C:P ratio	165	99	195	161	103	137
N:P ratio	10	18	9	10	20	7
Vaccinium myrtillus						
Carbon content (%)	51.9	48.0	36.8	51.8	51.4	35.2
Nitrogen content (%)	2.4	8.8	1.4	2.5	9.6	1.3
Phosphorus content (%)	0.23	0.45	0.20	0.22	0.46	0.17
$\delta^{13}C$	−29.2	−29.1	−30.2	−29.9	−28.3	−30.2
$\delta^{15}N$	−1.7	1.8	−1.6	−1.3	0.2	−1.6
C:N ratio	21.6	5.5	26.0	20.5	5.3	26.8
C:P ratio	228	107	187	237	112	211
N:P ratio	11	20	7	12	21	8

material from each plant–herbivore combination for chemical analysis (~50 mg for C and N analyses, ~200 mg for P analysis), which means that we do not have replicate-level information on chemistry. Total amounts of C, N and P converted to larval bodies and frass for each of the herbivore–plant combinations ($n = 1$) were calculated by multiplying dry biomass of larvae bodies and frass (*O. brumata*, $n = 5$; *E. autumnata*, $n = 6$) by the elemental content of the same material ($n = 1$). Total masses of N and P ingested per herbivore–plant combination ($n = 1$) were estimated as the sum of each element converted to both larvae and frass. To estimate the portion of ingested C allocated to respiration, we first multiplied the foliar C:N ratio by the estimated total mass of N ingested for all herbivore–plant combinations to estimate the total C ingested, then we subtracted the total mass of C in larval biomass bodies and frass from the mass of ingested C. Errors around mean values were propagated by quadrature of absolute errors for addition and subtraction, and quadrature of relative errors for division and multiplication.

The level of internal body C:N:P was measured as H, the homeostatic regulation coefficient (Sterner & Elser, 2002). H is calculated from the equation:

$$\log(\mathrm{C:N, C:P \text{ or } N:P})_{\mathrm{biomass}} = a + \frac{1}{H}\log(\mathrm{C:N, C:P \text{ or } N:P})_{\mathrm{plant}}$$

where $(\mathrm{C:N, C:P \text{ or } N:P})_{\mathrm{biomass}}$ are, respectively, the C:N, C:P or N:P ratio of elements in the herbivore bodies, measured directly from the pupae and larvae samples; $(\mathrm{C:N, C:P \text{ or } N:P})_{\mathrm{plant}}$ are, respectively, the C:N, C:P or N:P ratio of elements in the plant species, measured directly from plant material; and a is a constant. H varies between 0 and $+\infty$. Organisms with H values between 0 and 2 are considered non-homeostatic, between 2 and 4 as weakly homeostatic, and > 4 as strongly homeostatic. On occasion, H can take a high negative value, indicative of strong homeostasis. For each herbivore–plant combination, we calculated AD and ECI for C, and ECI for N and P. AD cannot be calculated for elements other than C in our experiment, because frass mixes both non-digested and excreted N and P. AD for C was calculated as:

$$\mathrm{AD} = \frac{\mathrm{C}_{\mathrm{ingested}} - \mathrm{C}_{\mathrm{frass}}}{\mathrm{C}_{\mathrm{ingested}}}$$

The ECI for the three elements C, N and P was calculated as:

$$\mathrm{ECI} = \frac{(\mathrm{C, N \text{ or } P})_{\mathrm{biomass}}}{(\mathrm{C, N \text{ or } P})_{\mathrm{ingested}}}$$

where X_{ingested} is the amount of the given element X ingested by the larvae during the experiment, X_{frass} is the amount of ingested X converted to frass, and X_{biomass} is the amount of ingested X accumulated in the biomass of the growing larvae bodies. The isotopic signatures (δ) were calculated as

$$\delta^y X = \left(\frac{\frac{{}^yX_{\mathrm{sample}}}{{}^zX_{\mathrm{sample}}}}{\frac{{}^yX_{\mathrm{standard}}}{{}^zX_{\mathrm{standard}}}} - 1 \right) \times 1000‰$$

where y is the unit mass of the least abundant (heavy) isotope, z is the unit mass of the abundant (light) isotope and X is the element of interest. The N standard is atmospheric air and the C standard is the Pee Dee Belemnite. The enrichment or discrimination factors (Δ) were calculated as

$$\Delta^y X = \delta^y X_{\mathrm{frass,body}} - \delta^y X_{\mathrm{diet}}$$

Statistical analyses

Differences in larval survival and body biomass growth were assessed with a univariate general linear model and a least significant difference *post hoc* test. Variables were transformed where necessary to conform to parametric assumptions. Relationships between plant and herbivore stoichiometry, herbivore growth and isotopic enrichment of herbivore products were assessed with a Spearman's rank correlation, which was selected because it made no assumptions about the underlying distribution of data.

Differences in the homeostatic regulation coefficient H between the two herbivores was assessed with a linear model regressing $(\mathrm{C:N, C:P \text{ or } N:P})_{\mathrm{biomass}}$ against $(\mathrm{C:N, C:P \text{ or } N:P})_{\mathrm{plant}}$ in interaction with herbivore species identity as an independent factor. Significance and confidence intervals (CIs) were calculated on the slope $1/H$, because using the inverse of the slope entails well-known statistical problems (Persson *et al.*, 2010).

Approximate digestibility and ECI for C, N and P were compared between the two herbivore species using ANCOVA analyses as previously recommended (Raubenheimer & Simpson, 1999). The dependent variable was $\mathrm{C}_{\mathrm{ingested}} - \mathrm{C}_{\mathrm{frass}}$ for AD, and $(\mathrm{C, N \text{ or } P})_{\mathrm{biomass}}$ for ECIs. The ingested amount of the corresponding element ($\mathrm{C, N \text{ or } P}_{\mathrm{ingested}}$ respectively) was used as a covariate in all ANCOVAs and herbivore species identity was used as the independent factor. Variables were transformed where necessary to conform to parametric assumptions.

Replicated boxes for each of the six herbivore–plant combinations (each box containing 20 larvae) were pooled together before chemical analyses. This means that each combination was represented by only one point in all statistical analyses. But what was lost in terms of number of replicates was gained in terms of precision, as each data point represents the mixed average of 120 larvae for *E. autumnata* (six boxes times 20 larvae) and 100 larvae for *O. brumata* (five boxes times 20 larvae).

Results

Patterns of herbivore growth and mortality

Epirrita autumnata displayed significantly lower rates of mortality compared with *O. brumata* (ANOVA, $F = 10.224$, d.f. = 1, $P < 0.001$; Fig. 1). Plant diet affected mortality rates of both herbivore species, with mortality higher among larvae raised on *B. pubescens* than among larvae raised on *B. nana* and *V. myrtillus*, although this difference was not statistically significant in the case of *O. brumata* (Fig. 1). Relative allocation to herbivore body production did not differ among herbivore species (Fig. 1) but displayed some signs of a dietary effect with a significantly lower body growth allocation among larvae raised on

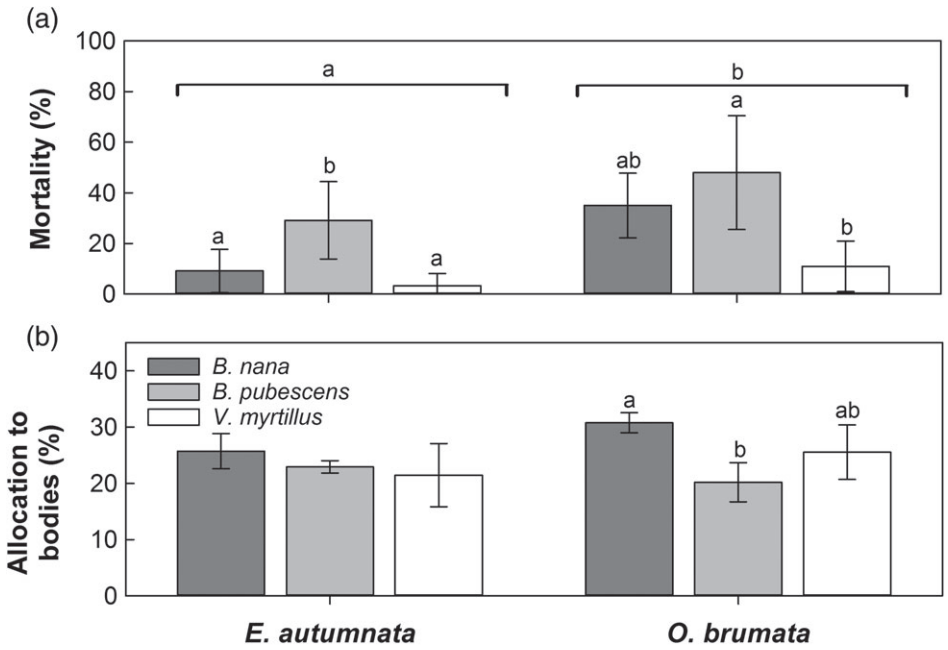

Fig. 1. Variation in herbivore mortality (a) and body biomass growth (b) by moth species and diet. Bars and error bars denote means and 95% confidence intervals ($n = 6$ for *Epirrita autumnata*, $n = 5$ for *Operophtera brumata*). Different letters above bars denote significant differences among categories.

B. pubescens than among those raised on *B. nana*, but only for *O brumata* (Fig. 1). The pattern of body biomass allocation among all herbivore larvae was significantly negatively correlated with plant dietary C content (SRC, correlation coefficient = −0.829, $P = 0.042$) and was closely related to survival, such that greater allocation to growth decreased survival (SRC, correlation coefficient = −0.943, $P = 0.005$).

Patterns of herbivore C, N and P allocation

The absolute quantity of C, N and P allocated to herbivore products (bodies, frass and respiration) was higher in both herbivore species fed on *B. nana* (Supporting information Fig. S1) but this was entirely explained by the generally higher ingestion rate of *B. nana* compared with *B. pubescens* and *V. myrtillus* (Supporting information Fig. S1). After accounting for differences in the total quantity of C, N and P ingested, relative differences in partitioning became minimal (Fig. 2). Ingestion rate varied widely between herbivore and plant species (Supporting information Fig. S1) but was not significantly related to plant dietary content or ratios of C, N or P. Considering individual herbivore products, there were no large differences in allocation to bodies or (in the case of C) respiration between herbivore species (Fig. 2, Supporting information Fig. S1), but *E. autumnata* allocated consistently greater amounts of C, N and P to frass than *O. brumata* (Fig. 2).

Stoichiometric constraints on herbivore C, N and P allocation

Both AD and ECI were linearly related to ingestion rate, with no clear effect of different plant species (Fig. 3). Across both herbivore species, AD for C was between 0.59 and 0.78, while ECI for C, N and P was constrained to values in the ranges 0.18–0.28, 0.67–0.83 and 0.46–0.62, respectively (Table 2). There was no significant variation between herbivore species for C and P efficiencies (AD for C, $P = 0.11$; ECI for C, $P = 0.47$; ECI for P, $P = 0.10$). By contrast, ECI for N was significantly higher in *O. brumata* than in *E. autumnata* ($P = 0.027$) (Fig. 3). *Operophtera brumata* consistently maintained greater homeostatic control of body C, N and P, across the wide variation in physico-chemical properties of plant diet supplied (Table 2) than was the case for *E. autumnata*, with H values of 13.03 (95% CIs for $1/H$: −1.36–1.52) versus 2.67 (−0.62–1.37) for C:N, 27.75 (−0.92–0.99) versus 3.09 (−0.37–1.02) for C:P, and − 10.32 (−4.93–4.74) versus 2.06 (−3.56–4.53) for N:P, but lower overall body N and P content than *E. autumnata* (Table 2), although none of these differences was statistically significant (Fig. 4).

Enrichment of ^{13}C and ^{15}N in herbivore products

Overall mean enrichment values of ^{13}C in bodies and frass were 0.3 ± 0.3 and -0.4 ± 0.2, respectively, while mean enrichment values in bodies and frass for ^{15}N were 2.7 ± 0.5 and 0.3 ± 0.5, respectively (Fig. 5). The dominant control over the enrichment rate was the isotopic level of the plant species ingested. Specifically, herbivores fed on material with more negative ^{15}N signatures produced bodies significantly more enriched in ^{15}N (SRC, correlation coefficient = −0.83, $P = 0.042$) but less enriched in ^{13}C (SRC, correlation coefficient = 0.83, $P = 0.042$), and frass more enriched in ^{15}N SRC, correlation coefficient = −0.89, $P = 0.019$) (Table 3). The pattern of frass ^{13}C enrichment was unrelated to the ^{15}N

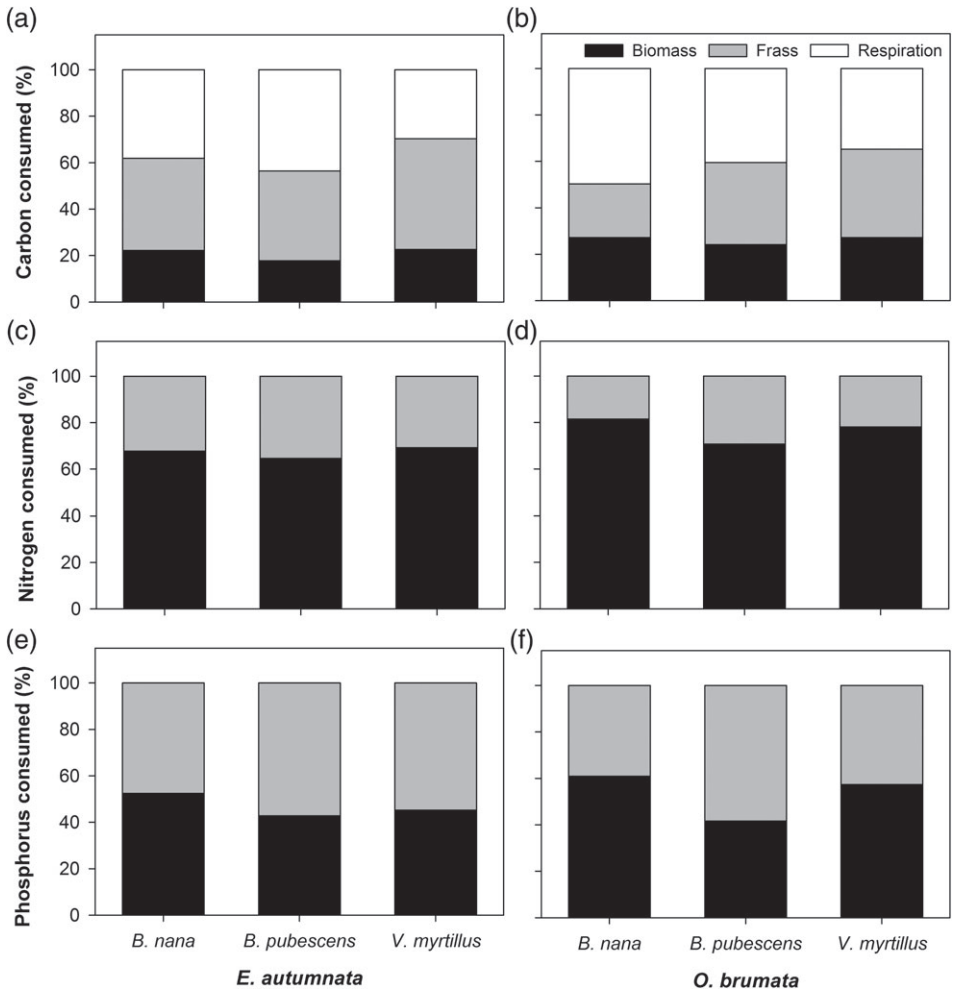

Fig. 2. Relative variation in element partitioning to different products by moth species and diet. Bars represent the mean of each herbivore species × plant species combination ($n = 1$).

signature in the source material but did significantly increase with more negative ^{13}C levels in food (SRC, correlation coefficient = −0.94, $P = 0.005$) (Table 3). Litter chemical quality played no clear role in the patterns of enrichment observed (Table 3).

Discussion

Our observations of elemental partitioning and stoichiometry by two geometrid moth species provide a useful first outline of the pathways for ingested material after defoliation events, and some of the mechanisms regulating these pathways. The patterns observed should be interpreted with caution, given the low level of replication, but raise a number of potentially important issues and questions which merit further study.

Defoliation during moth outbreak has an immediately severe negative impact on the C sink strength of subarctic birch forests, impeding photosynthetic C uptake by as much as 90% in the year of the outbreak (Heliasz *et al.*, 2011; Olsson *et al.*, 2017) and causing reduced growth and enhanced mortality in the years afterwards (Tenow & Bylund, 2000; Tenow *et al.*, 2004). Moreover, insect deposits decompose more rapidly than senesced litter in the study system (Kristensen *et al.*, 2018). We identify another process further reducing the short-term C sink during the outbreaks: between 30% and 50% of material consumed over a month was rapidly released as CO_2 via respiration depending on the herbivore species and diet (Fig. 2). The pathway for this material in a non-outbreak year, as leaf litterfall was transferred to the ground, may also eventually have resulted in release of CO_2, via microbial breakdown, but to a lesser degree and over a much longer timescale given the recalcitrant plant material and abiotic conditions, which impede decomposition (Aerts, 1997; Sjögersten & Wookey, 2004; Zhang *et al.*, 2008).

Our observations indicate that the patterns of internal processing of elements by the two herbivore species studied was affected mainly by the quantity of element ingested (Figs. 4 and 5), with little apparent effect of plant species-specific variation in chemical or physical traits. These findings should be

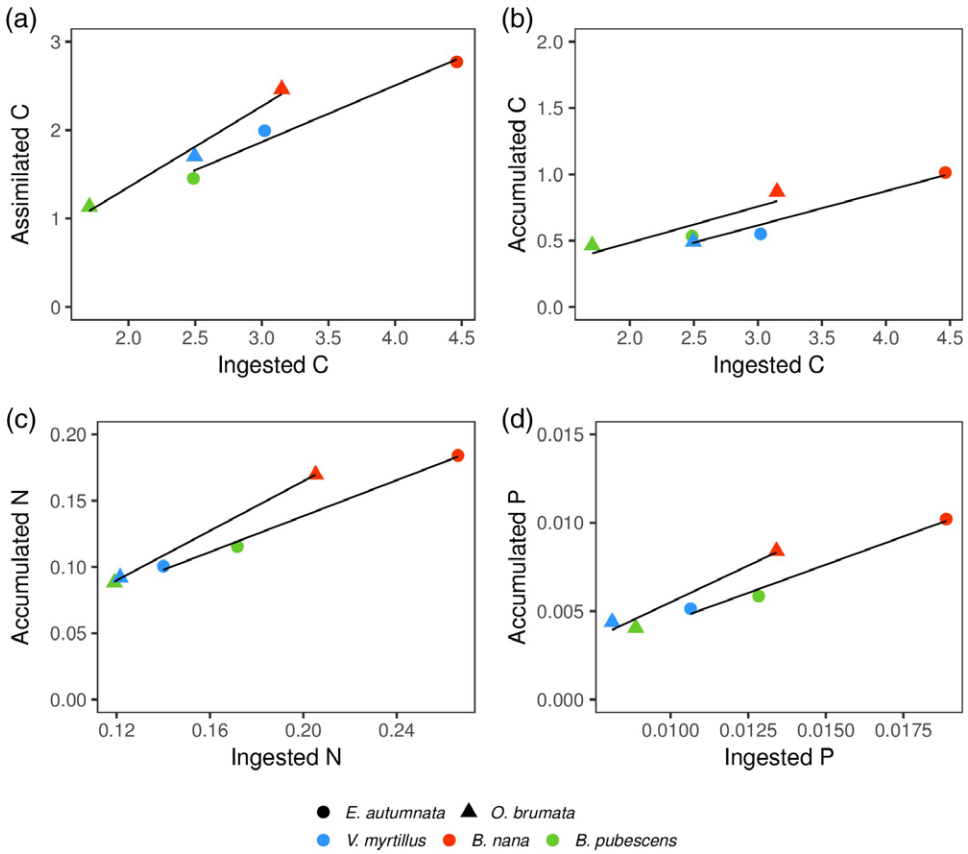

Fig. 3. Differences among moth species in efficiency of assimilation of ingested C (apparent digestibility) (a), and transformation of ingested C (b), N (c), and P (d) into body mass (efficiency of conversion of ingested food). Each dot represents the mean of each herbivore species × plant species combination. Red, *Betula nana*; green, *Betula pubescens*; blue, *Vaccinium myrtillus*; circles, *Epirrita autumnata*; triangles, *Operophtera brumata*. Lines represent linear regressions through species-specific data ($n = 3$).

Table 2. Assimilation efficiencies (approximate digestibility, AD) and efficiencies of conversion of ingested food (ECI) for all herbivore–plant combinations and for the three elements C, N and P.

	Epirrita autumnata			*Operophtera brumata*		
	Carbon	Nitrogen	Phosphorus	Carbon	Nitrogen	Phosphorus
Betula pubescens						
AD	0.59	–	–	0.66	–	–
ECI	0.21	0.67	0.46	0.27	0.74	0.46
Betula nana						
AD	0.62	–	–	0.78	–	–
ECI	0.23	0.69	0.54	0.28	0.83	0.63
Vaccinium myrtillus						
AD	0.66	–	–	0.68	–	–
ECI	0.18	0.72	0.48	0.20	0.76	0.54

interpreted with caution given, first, that the larvae selected had spent their first and second instars feeding in nature and so their stoichiometry may partly still reflect this early stage and, second, that the plant material supplied was collected at the same time but at different phenological stages because they follow different growth trajectories during the growth season. In particular, *B. pubescens* tends to undergo bud-burst earlier than the other species so the *B. pubescens* leaves sampled had probably progressed further along their maturation trajectory, which is characterised by a decrease in nutritional quality (Ayres & MacLean, 1987; Hanhimäki *et al.*, 1995), so this could explain the surprisingly low survival and consumption among larvae raised on *B. pubescens* foliage in our experiment. Previous work with laboratory-raised larvae and phenologically matched

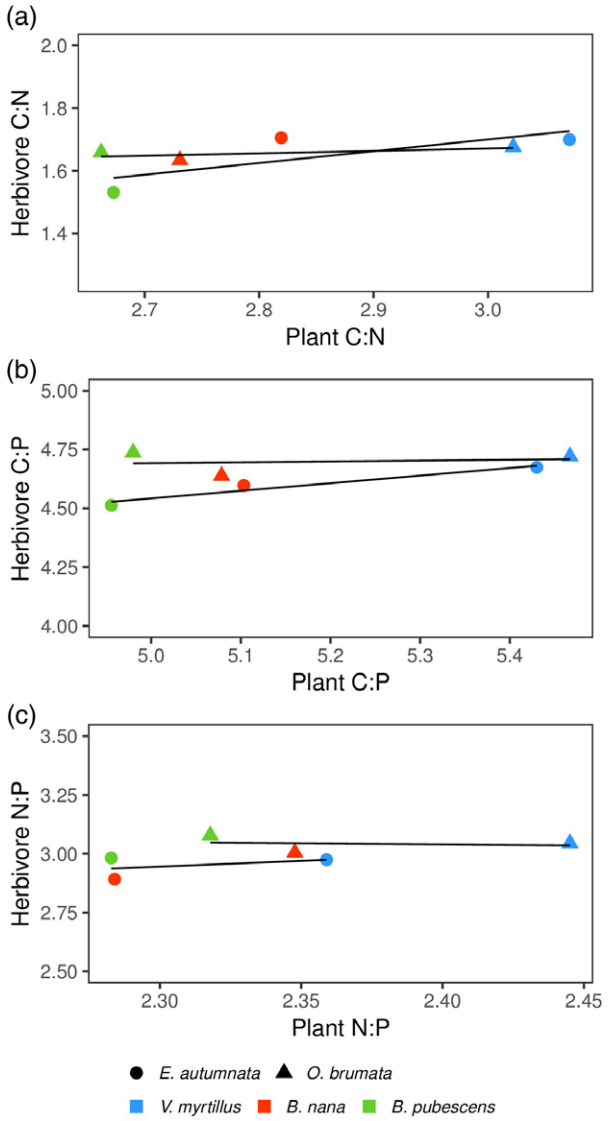

Fig. 4. Comparing stoichiometric homeostasis between moth species. *H*, the inverse of the slope on the log–log scale, is a measure of homeostasis (the higher *H*, the more homeostatic). Each dot represents the mean of each herbivore species × plant species combination. Red, *Betula nana*; green, *Betula pubescens*; blue, *Vaccinium myrtillus*; circles, *Epirrita autumnata*; triangles, *Operophtera brumata*. Lines represent linear regressions through species-specific data ($n = 3$).

plant material found little evidence for difference in ingestion, growth and/or mortality rates indicative of dietary specialisation (Neuvonen *et al.*, 1987; Ruohomäki & Haukioja, 1992). However, the leaf phenological stage sampled is representative of the time period (June) during which moth larvae herbivory rates peak, so our observations are likely to be representative of herbivory in natural systems. If this lack of any strong diet quality effect on herbivore nutrient outputs via frass and bodies is representative of other ecosystems and herbivores, then the challenge of incorporating herbivore activity into biogeochemical models is considerably simplified (Throop *et al.*, 2004; Ostle *et al.*, 2009).

In our study, *E. autumnata* displayed a lower level of stoichiometric homeostasis compared with *O. brumata* (Fig. 5), although these observations should be interpreted with caution given the low level of replication. This greater capacity to maintain optimal body elemental ratios could translate into important differences in food preference and patterns of outbreak between these herbivore species, which merit further research. While much previous work has focused on the biogeochemical importance and impacts of frass (e.g. Hunter, 2001), we find that both species, but particularly *O. brumata*, were highly efficient at incorporating ingested N into body mass and excreted relatively little ingested N via frass (Fig. 2, Fig. 4). Therefore, a greater research focus on the ecological and biogeochemical impacts of deposition of herbivore bodies during and immediately after outbreaks is merited. Indeed, studies in other systems have already demonstrated how important the transfer of carbon and nutrients via bodies may be for nutrient cycling (Yang, 2004; Kos *et al.*, 2017).

A predictive understanding of the patterns in, and underlying drivers of, natural variation in ^{13}C and ^{15}N is necessary to fulfil the promise of stable isotopes as powerful tools to map and probe trophic networks in nature. Previous work indicates that the degree of ^{13}C and ^{15}N discrimination from primary producer to consumer may be linked with diet (Webb *et al.*, 1998; Vanderklift & Ponsard, 2003; Caut *et al.*, 2009), feeding mode (McCutchan *et al.*, 2003) and/or herbivory physiology [e.g. recycling internal N stores (Hobson & Clark, 1992), number of life stages necessitating metamorphosis (Patt *et al.*, 2003)]. In this study, we observed strong differences among herbivore species in terms of discrimination patterns (Fig. 5), but without more detailed information about herbivore physiology it remains difficult to ascertain the underlying mechanisms responsible. We found that certain combinations of diet and herbivore produced exceptionally high enrichment of ^{15}N (*E. autumnata* feeding on *V. myrtillus* and *B. pubescens*, *O. brumata* feeding on *B. pubescens*) and ^{13}C (both herbivores feeding on *B. nana* and *V. myrtillus*), but there was little evidence for any effects of plant species or dietary chemical quality on ^{13}C and ^{15}N discrimination in herbivore bodies and frass (Fig. 5). In line with Caut *et al.* (2009), however, enrichment was consistently related to ^{13}C and ^{15}N levels of the dietary material. Similar to the findings of our study, the only published dataset we could find for herbivorous insect frass discrimination factor (Wehi & Hicks, 2010) also found that frass discrimination factors were more strongly related to isotope signatures of the diet than were body discrimination factors, which are the most commonly used factors in mixing models (Caut *et al.*, 2009). Hence, frass discrimination factors may be a promising and more accurate new assay for examining trophic relationships, and deserves more attention in future. Broadly in line with our findings, Spence and Rosenheim (2005) concluded that our ability to predict isotopic enrichment based upon diet and herbivore traits is so limited that enrichment factors may have to be directly calculated for each trophic linkage of interest, rather than generalised from literature values. In this context, our study provides enrichment factors for a key plant–herbivore complex

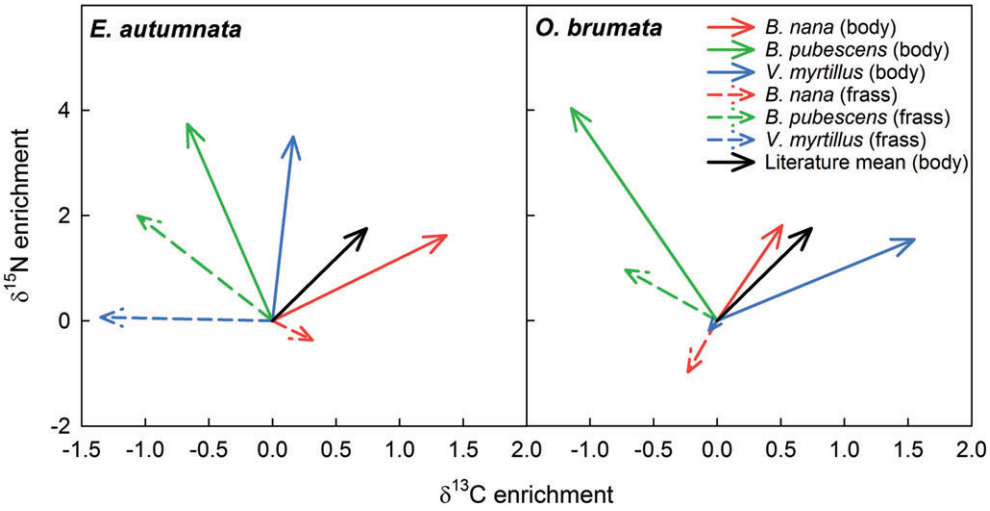

Fig. 5. Isotopic enrichment of various products from moth species raised on different plant species. The literature mean is derived from Spence and Rosenheim (2005) and represents 27 terrestrial arthropod - plant pairs collected using a wide array of methods and herbivore life stages. The source of the arrows denotes the original $\delta^{13}C$ and $\delta^{15}N$ of the plant species ingested standardised to zero for all plant species. The arrow heads denote the change in $\delta^{13}C$ and $\delta^{15}N$ within bodies and frass relative to the plant species ingested.

Table 3. Results of a Spearman's rank correlation between chemical properties of foliar diet and isotopic enrichment in different herbivore products.

Foliar properties	^{13}C enrichment		^{15}N enrichment	
	Bodies	Frass	Bodies	Frass
C content	0.87 (−0.86)	0.07 (−0.77)	0.87 (0.86)	0.33 (0.49)
N content	0.16 (−0.67)	0.96 (0.03)	0.16 (0.67)	0.47 (0.37)
C:N ratio	0.16 (0.66)	0.96 (−0.03)	0.16 (−0.66)	0.47 (−0.37)
$\delta^{13}C$	0.40 (−0.43)	**0.005 (−0.94)**	0.40 (0.43)	0.27 (0.54)
$\delta^{15}N$	**0.04 (0.83)**	0.16 (0.66)	0.04 (−0.83)	**0.02 (−0.89)**

Data are *P*-values (correlation coefficient). Significant results are highlighted in bold.

(two herbivore and three plant species) which has major impacts on ecology and biogeochemical cycling across large areas of the Fennoscandian subarctic.

Conclusion

The aims of this manuscript were broadly two-fold: first, to describe partitioning of C, N and P by two widespread insect herbivore species in the Fennoscandian subarctic; and second, to quantify how the pattern of partitioning and the chemical composition of herbivore products were affected by the plant species and chemical content of the diet. We highlight several patterns and trends which merit further investigation in more extensive laboratory trials and field surveys. First, relatively large quantities of N and P were allocated to bodies rather than to frass, indicating that the quantity and chemical quality of herbivore bodies deposited during and immediately after moth outbreaks may be of greater importance than previously appreciated for understanding longer-term ecosystem impacts. Second, the efficiency of absorption of ingested materials and subsequent allocation to bodies, frass and respiration did not differ strongly and consistently between moth species and plant species ingested. This apparent lack of sensitivity to species-specific variation could simplify attempts to model biogeochemical impacts of moth herbivore across the region. Together, these results have important implications for how projected shifts in the range and population dynamics of these herbivorous moth species across the Fennoscandian subarctic will impact biogeochemical cycling in the region. Further work using controlled mesocosms with greater sample sizes, laboratory-raised larvae and phenologically matched leaf diets is required to reinforce and extend these findings.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Absolute variation in element partitioning to different products by moth species and diet. Bars represent the mean of each herbivore species × plant species combination ($n = 1$).

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