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

Running title: Stoichiometry of Arctic moth herbivores.

Authors: Daniel B. Metcalfe[1,2], Mehdi Cherif[2], Jane U. Jepsen[3], Ole Petter L. Vindstad[4], Jeppe Å. Kristensen[1], and Ulrika Belsing[1].

Affiliations: [1]Department of Physical Geography and Ecosystem Science, Lund University, SE 223-62, Lund, Sweden; [2]Department of Ecology and Environmental Science, Umeå University, SE 901-87 Umeå, Sweden; [3]Department of Arctic Ecology, Norwegian Institute for Nature Research (NINA), Tromsø, Norway; 4 University of Tromsø – The Arctic University of Norway, Department of Arctic and Marine Biology, Framstredet 39, N-9037 Tromsø, Norway.

Corresponding author: Daniel Metcalfe; Department of Physical Geography and Ecosystem Science, Lund University, SE 223-62, Lund, Sweden; Tel.: +46 (0)72 205 2795; Email: dbmetcalfe@gmail.com; ORCID: https://orcid.org/0000-0001-8325-9269.

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

3. Overall, 23%, 70% and 48% of ingested C, N and P was allocated to bodies respectively, rather than frass and (in the case of C) respiration. *O. brumata* consistently maintained more constant body stoichiometric ratios of C, N and P than *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 our ability to predict the amount and stoichiometry of frass and bodies generated after geometrid outbreaks.

Keywords: consumer-driven nutrient recycling, ecological stoichiometry, subarctic birch forest, geometrid moth, homeostasis, stable isotope.

Introduction

The key nutrients limiting plant growth in high-latitude forests are nitrogen (N) and, in some cases, phosphorus (P) (Giesler et al. 2004, Vitousek & Howarth, 1991), but the effects of herbivores on ecosystem-level availability of these nutrients remain poorly understood (Bardgett & Wardle 2003, Grüning et al. 2017, Hartley and Jones 2004, Hunter 2001, Sitters et al. 2017). Most research on the ecosystem effects of herbivores have focused to date on large mammals (Pastor et al. 1988, Augustine and McNaughton 1998, Olofsson et al. 2004). By comparison with mammals, less is known about the role of insect herbivores, though available studies suggest that insect population outbreaks can exert major impacts on ecosystem structure and function (Kaukonen et al. 2013, Metcalfe et al. 2013, 2016, Volney & Fleming 2000). 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 Ahti) forests across Fennoscandia at regular intervals (Haukioja 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 autumnal moth (*Epirrita autumnata*) and winter moth (*Operophtera brumata*). The spatio-temporal patterns of moth outbreaks and defoliation (Ims et al. 2004, Jepsen et al. 2008, Tenow et al. 2007) and observations of the end results of defoliation on vegetation and soils (Jepsen et al. 2013, Kaukonen et al. 2013, Kristensen et al. 2018, Parker et al. 2016, Saravesi et al. 2015) have been studied. By comparison, there exist limited information about the intermediate steps and underlying mechanisms linking macro-scale observations of outbreaks to the longer-term consequences for ecosystem biogeochemistry. In part, this data paucity 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 2001, Hunter & Price 1992, 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 provide 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 (Ben-David & Schell 2001, Caut et al. 2008, 2009, Gannes et al. 1997).

The quantity of ingested C, N and P diverted to different herbivore products is the end result of several steps (Scriber & Slansky 1981, Waldbauer 1968). 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 inter-linked 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 defense compounds may suppress AD and / or ECI through various mechanisms (e.g.: altered gut passage time, elevated respiratory rates, Berner 2005, Clissold et al. 2009, Cresswell et al. 1992,

92 Raubenheimer & Simpson 1999). The extent of homeostatic control over internal C, N, P ratio
93 could prove useful in predicting the scale and spatial pattern of potential range shifts with
94 climate change (Gonzalez et al. 2010, Ward and Masters 2007). If these predictions hold across a
95 diversity of herbivore types and host plant species they would potentially provide a framework to
96 link plant chemical traits to herbivore-mediated nutrient fluxes, and anticipate differences in
97 responses among herbivores to environmental changes, thereby facilitating improved integration
98 of herbivore activity into global models (Ostle et al. 2009, Throop et al. 2004).

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

111

112 **Materials and methods**

113 ***Study system***

The study area was around Tromsø in northern Norway (69°38’56.6’’N 18°57’17.1’’E) that 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 understory 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 from 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 sampled *O. brumata*. Both moth species can be subject to parasitoids (Virtanen and Neuvonen 1999), but the occurrence of parasitoids and prevalence of larval parasitism were not surveyed during sampling, though 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 hours and weighed separately. Thus, the larvae were raised on a natural diet for one-two instars before inclusion in the experiment. For *E. autumnata,* 20 live larvae were placed within 6 boxes each filled with fresh leaves from only one plant species: *B. pubescens*, *B. nana* or *V. myrtillus* (3 plant species × 6 replicates = 18 boxes total). For *O. brumata*, 20 live larvae were placed within 5 boxes each filled with fresh leaves from only one plant species: *B. pubescens*, *B. nana* or *V. myrtillus* (3 plant species × 5 replicates = 15 boxes 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 8 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 hours and then weighed. After one month, the pupae and unpupated larvae from each box were counted, dried at 60 °C for 48 hours 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 initiation 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 which were either 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” above) 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 6 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 analyzer (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 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 C, N and P converted to larval bodies and frass for each of the herbivore-plant combinations (n = 1) was calculated by multiplying dry biomass of larvae bodies and frass (n = 5 for *O. brumata* and 6 for *E. autumnata*) by the elemental content of the same material (n = 1). Total mass of N and P ingested per herbivore-plant combination (n = 1) was 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 foliar C:N ratio by the estimated total mass of N ingested for all herbivore-plant combinations to estimate total C ingested, then secondly, 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:

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

where $(C:N,\ C:P \text{ or } N:P)_{biomass}$ 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; $(C:N,\ C:P \text{ or } N:P)_{plant}$ 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 above 4 as strongly homeostatic. On occasion, H can take a high negative value, indicative of strong homeostasis. For each herbivore-plant combination, we calculated assimilation efficiency (AD) and efficiency of conversion of ingested food (ECI) for C, and efficiency of conversion of ingested food (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:

$$AD = \frac{C_{ingested} - C_{frass}}{C_{ingested}}$$

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

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

where $X_{ingested}$ is the amount of the given element X ingested by the larvae during the experiment, X_{frass}, the amount of ingested X converted to frass, and $X_{biomass}$ the amount of

ingested X accumulated in the biomass of the growing larvae bodies. The isotopic signatures (δ) were calculated as

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

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

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

Statistical analyses

Differences in larval survival and body biomass growth were assessed with a univariate general linear model (GLM) and an LSD posthoc 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 $(C{:}N,\ C{:}P \text{ or } N{:}P)_{biomass}$ against $(C{:}N,\ C{:}P \text{ or } N{:}P)_{plant}$ in interaction with herbivore species identity as an independent factor. Significance and confidence

intervals were calculated on the slope $\frac{1}{H}$, because using the inverse of the slope entails well-known statistical problems (Persson et al, 2010).

AD and ECI for C, N and P were compared between the two herbivore species using ANCOVA analyses as recommended (Raubenheimer & Simpson 1999). The dependent variable was $C_{ingested}$-C_{frass} for AD, and (C, N or P)$_{biomass}$ for ECIs. The ingested amount of the corresponding element (C, N or $P_{ingested}$ 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 6 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, since each data point represents the mixed average of 120 larvae for *E. autumnata* (6 boxes times 20 larvae) and 100 larvae for *O. brumata* (5 boxes times 20 larvae).

Results

Patterns of herbivore growth and mortality

E. autumnata displayed significantly lower rates of mortality than *O. brumata* (Fig. 1, ANOVA, $F = 10.224$, d.f = 1, $P < 0.001$). Plant diet affected mortality rates of both herbivore species, with mortality higher among larvae raised on *B. pubescens* compared to 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 *B. pubescens* than *B. nana*, but only for *O brumata* (Fig. 1). The pattern of body biomass allocation among all herbivore larvae was significantly negatively correlated to 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 1) but this was entirely explained by the generally higher ingestion rate of *B. nana* compared to *B. pubescens* and *V. myrtillus* (Supporting information Fig. 1). 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. 1) 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. 1), 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 were constrained to values between 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 *E. autumnata* ($p = 0.027$) (Fig. 3). *O. 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 *E. autumnata* with H values of 13.03 (95% confidence intervals for $\frac{1}{H}$ = -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 were statistically significant (Fig. 4).

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

Overall mean enrichment of ^{13}C in bodies and frass was 0.3 ± 0.3 and -0.4 ± 0.2 respectively, while mean enrichment in bodies and frass for ^{15}N was 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 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 give 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 immediate 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 et al. 2004, Tenow & Bylund 2000). 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 – 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 transferred to the ground, may also have resulted eventually in release of CO_2, via microbial breakdown, but to a lesser degree and over a much longer time-scale 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 & 5), with little apparent effect of plant species-specific variation in chemical or physical traits. These findings should be interpreted with caution given, first, that the larvae selected had spent their first-second instar feeding in nature and so their stoichiometry may partly still reflect this early stage and, second, the plant material supplied was collected at the same time but 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.*

318 *pubescens* leaves sampled had likely progressed further along their maturation trajectory, which
319 is characterized by a decrease in nutritional quality (Ayres & MacLean 1987, Hanhimäki et al.
320 1995), so this could explain the surprisingly low survival and consumption among larvae raised
321 on *B. pubescens* foliage in our experiment. Previous work with lab-raised larvae and
322 phenologically matched plant material found little evidence for difference in ingestion, growth
323 and/or mortality rates indicative of dietary specialization (Neuvonen et al. 1987, Ruohomäki &
324 Haukioja 1992). However, the leaf phenological stage sampled is representative of the time
325 period (June) during which moth larvae herbivory rates peak, so our observations are likely to be
326 representative of herbivory in natural systems. If this lack of any strong diet quality effect on
327 herbivore nutrient outputs via frass and bodies is representative of other ecosystems and
328 herbivores, then the challenge of incorporating herbivore activity into biogeochemical models is
329 considerably simplified (Ostle et al. 2009, Throop et al. 2004).

330 In our study, *E. autumnata* displayed a lower level of stoichiometric homeostasis
331 compared to *O. brumata* (Fig. 5), though these observations should be interpreted with caution
332 given the low level of replication. This greater capacity to maintain optimal body elemental
333 ratios could translate into important differences in food preference and patterns of outbreak
334 between these herbivore species, which merit further research. While much previous work has
335 focused on the biogeochemical importance and impacts of frass (e.g: Hunter 2001), we find that
336 both species, but particularly *O. brumata*, were highly efficient at incorporating ingested N into
337 body mass and excreted relatively little ingested N via frass (Fig. 2, Fig. 4). Therefore, a greater
338 research focus on the ecological and biogeochemical impacts of deposition of herbivore bodies
339 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 (Kos et al. 2017, Yang 2004).

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 (Caut et al. 2009, Vanderklift and Ponsard 2003, Webb et al. 1998), feeding mode (McCutchan et al. 2003) and / or herbivory physiology (e.g.: recycling internal N stores, Hobson and Clark 1992, number of life stages necessitating metamorphosis, Patt et al. 2003). In this study, we observe 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 find 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 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 and Hicks, 2010) also found that frass discrimination factors were more strongly related to isotope signatures of the diet than the 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 future attention. Broadly in line with our findings, Spence and

Rosenheim (2005) conclude 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 generalized from literature values. In this context, our study provides enrichment factors for a key plant-herbivore complex (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. 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 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 strongly and consistently differ 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 are required to reinforce and extend these findings.

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Figure Legends

Figure 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 E. autumnata, n = 5 for O. brumata).Different letters above bars denote significant differences among categories.*

Figure 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).*

Figure 3) Differences among moth species in efficiency of assimilation of ingested C (AD, Apparent Digestibility) (a), and transformation of ingested C (b), N (c), and P (d) into body mass (ECI, Efficiency of Conversion of Ingested food). *Each dot represents the mean of each herbivore species × plant species combination. Red = B. nana, green = B. pubescens, blue = V. myrtillus. Circles: E. autumnata, triangles: O. brumata. Lines represent linear regressions through species-specific data (n = 3).*

Figure 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 = B. nana, green = B. pubescens, blue = V. myrtillus. Circles: E. autumnata, triangles: O. brumata. Lines represent linear regressions through species-specific data (n = 3).*

Figure 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*

standardized 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.

588 **Tables**

	E. autumnata			*O. brumata*		
	Leaves	Bodies	Frass	Leaves	Bodies	Frass
B. 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
B. 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
V. 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

589 ***Table 1) Mean chemical properties of plant leaves and products from herbivores raised***

590 ***exclusively on the leaves of the selected plant species.***

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	E. autumnata			*O. brumata*		
	Carbon	Nitrogen	Phosphorus	Carbon	Nitrogen	Phosphorus
B. pubescens						
AD	0.59	-	-	0.66	-	-
ECI	0.21	0.67	0.46	0.27	0.74	0.46
B. nana						
AD	0.62	-	-	0.78	-	-
ECI	0.23	0.69	0.54	0.28	0.83	0.63
V. myrtillus						
AD	0.66	-	-	0.68	-	-
ECI	0.18	0.72	0.48	0.20	0.76	0.54

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

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)
δ^{13}C	0.40 (-0.43)	**0.005 (-0.94)**	0.40 (0.43)	0.27 (0.54)
δ^{15}N	**0.04 (0.83)**	0.16 (0.66)	0.04 (-0.83)	**0.02 (-0.89)**

Table 3) Results of a Spearman's Rank Correlation between chemical properties of foliar diet and isotopic enrichment in different herbivore products. *Values represent p value (correlation coefficient). Significant results are highlighted in bold.*