Sources of variation in small rodent trophic niche: new insights from DNA metabarcoding and stable isotope analysis

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Intraspecific competition for food is expected to increase the trophic niche width of consumers, defined here as their diet diversity, but this process has been little studied in herbivores. Population densities of small rodents fluctuate greatly, providing a good study model to evaluate effects of competition on trophic niche. We studied resource use in five arctic small rodent populations of four species combining DNA metabarcoding of stomach contents and stable isotope analysis (SIA). Our results suggest that for small rodents the most pronounced effect of competition on trophic niche is due to increased use of secondary habitats and to habitat-specific diets, rather than an expansion of trophic niche in primary habitat. DNA metabarcoding and SIA provided complementary information about the composition and temporal variation of herbivore diets. Combing these two approaches requires caution, as the underlying processes causing observed patterns may differ between methodologies due to different spatiotemporal scales.

Keywords: herbivore; tundra food web; habitat use; trophic niche width; diet diversity; competition

1. Introduction

Intraspecific competition is often linked to an increase of a populations' trophic niche width (1, 2). We here refer to trophic niche as a part of the multidimensional ecological niche space of a population (3, 4), defined by food resource use. Trophic niche width thus describes the size of trophic niche and can be measured as diversity of used food resources (5-7). While a link between intraspecific competition and trophic niche width may exist for many consumers, the few studies that have investigated this relationship in herbivores suggest that different mechanisms may come into play (8-11). High herbivore population density may directly induce a narrowing of the trophic niche due to reduced plant species richness under a regime of intense grazing (9, 11). In addition, competition may indirectly affect trophic niche width. Increased use of secondary habitats at higher population densities, i.e. an increase in *habitat* niche width (defined analogously to trophic niche width, see above) has been documented in herbivores (12, 13), although also contradictory examples exist (14). As herbivore diets often differ between habitats (15, 16), an increase in a population's habitat niche width may consequently increase its trophic niche width. However, it is clear that current understanding of processes linking competition and herbivore trophic niche width and composition is incomplete.

In arctic and sub-arctic areas, the structure and dynamics of terrestrial food webs are largely shaped by high-amplitude population cycles of herbivorous lemming and vole populations (17-20). Such density fluctuations, also found outside the Arctic (21, 22), make small rodents a very well suited model group to investigate the consequences of competition on trophic niche. Several authors have hypothesized that during peaks of population density the availability of high-quality food for small rodents is limited, leading potentially to a change in population trophic niche (23-25). On the other hand, numerous studies have assumed that small rodents do not change the taxonomic composition of their diet during population density peaks (26-28). Still, only a handful of studies have evaluated changes of

90 small rodent food habits during population peaks (29-31). Population density of small rodents
91 has, however, been related to expansion of habitat use (13, 32-34). Nevertheless, the
92 relationship between habitat use and diet remains poorly understood in most small rodent
93 species (35). For instance, some studies have indicated that food availability is an important
94 determinant of small rodent food selection (36, 37), whereas others have found rather small
95 differences in small rodent diets among habitats in spite of differences in food availability
96 (38-40). Therefore, while competition may lead to an increase in habitat niche width in small
97 rodents, how this is reflected in the trophic niche remains little explored.
98 The current lack of knowledge about small rodent diets is mainly due to
99 methodological limitations, as microhistological studies on rodent stomach or feces content
100 are both taxonomically relatively imprecise and tedious to conduct (41). DNA metabarcoding,
101 i.e. simultaneous identification of multiple taxa from a sample containing a mixture of DNAs
102 by means of high-throughput sequencing of a carefully selected part of the genome (42, 43),
103 has recently opened up possibilities to analyze herbivore stomach contents with increased
104 taxonomic precision (41, 44-47). While DNA metabarcoding yields detailed information on
105 the content of the latest meal, long term resource use can be assessed using stable isotopes of
106 carbon (C) and nitrogen (N) (48, 49). Ratios of C^{13}/C^{12} and N^{14}/N^{15} (denoted as $\delta^{13}C$ and
107 $\delta^{15}N$) in a consumer's tissue reflect those of its food sources in a predictable manner (50, 51),
108 and thus integrate information on its multidimensional trophic niche into fewer dimensions
109 (e.g., bivariate when two isotopes are used). Consequently, isotopic ratios of a population can
110 be described as an isotopic niche, which size can be used to assess niche width (52-54).
111 Combined analyses of stomach contents and stable isotopes yield higher taxonomic precision
112 and wider timeframe, thus providing complementary insights unavailable through one method
113 alone (55-58). To our knowledge, (58, 59) are the only ones to date who have attempted to
114 combine stable isotope analysis and DNA-based methodology, using PCR-based taxon

identification. They found this approach to be a powerful combination, but suggest that high-throughput sequencing, as is used in DNA metabarcoding approaches, should open for further possibilities (58, 59).

Here, we combine the use of DNA metabarcoding with stable isotope analysis to investigate the relationships between population density, habitat niche and trophic niche. We also aimed to evaluate the possibilities and challenges related to the combined use of these methods for herbivore diet studies in particular. Specifically, we assessed the impacts of intraspecific competition on small rodent population trophic niche, evaluating both a) direct effects within the primary habitat and indirect effects mediated through changes in habitat use and b) trophic niche width and its composition. We always refer to the realized niche of a population (3, 4) and consider niche width as diversity of resource use, taking into account both the number of resources and relative intensity of their use (5-7). We used data from four arctic small rodent species from five populations and three distant study areas (see Table 1), across various plant communities and densities of small rodents. Assuming that an increase in population density leads to an increase in intraspecific competition, we hypothesized that it could in turn lead to H1) changes in the populations' trophic niche width and composition and/or H2) an increased heterogeneity of habitat use, i.e. wider habitat niche. We further hypothesized that H3) the composition of the trophic niche would differ between habitats reflecting food availability and hence H4) an increase of habitat niche width would lead to a wider trophic niche.

2. Material and Methods

2.1. Study areas

The data were collected in three different Arctic **study areas**; Finnmark in north-eastern Norway (70° N, 27-30° E) at the border of the sub-arctic and low-arctic zones, low-arctic

Nenetsky Ridge in Nenetsky Autonomous Okrug, Russia (68° 18' N, 53° 18' E) and high-arctic Bylot Island, Nunavut, Canada (73° 9' N, 79° 59' W) (Figure 1). More than one small rodent species are found at each study area, and most of them exhibit cyclic high-amplitude population dynamics (60-62). In the Finnmark study area, the data were collected from three different **study sites** separated by 40 to 60 km; Ifjordfjellet (IF), Vestre Jakobselv (VJ) and Komagdalen (KO) (Figure 1), whereas at Nenetsky and Bylot Island, all samples were collected in an area within a radius of 5 km. All data collection was done during snow-free period.

In the Finnmark study area, the data were collected in three habitats; dwarf-shrub heaths (primary habitat for grey-sided vole, *Myodes rufocanus*), willow-thicket meadow mosaics (hereafter called meadows, primary habitat for tundra vole, *Microtus oeconomus,* hereafter denoted as *M. oeconomus*$_{(\text{Finnmark})}$) and shrubby wetlands. While none of these habitats can be defined as an obvious primary habitat for Norwegian lemming *Lemmus lemmus,* the species is more abundant in the heath and wetland habitats than in the meadow habitat. We chose to assign heath as the "primary habitat" for *L. lemmus* in this study, as we had a very low sample size for the wetland habitat. In Nenetsky, similar meadows, inhabited by a *M. oeconomus* population (hereafter denoted as *M. oeconomus*$_{(\text{Nenetsky})}$), were sampled. On Bylot Island, data were collected in wetland (primary habitat for brown lemming *Lemmus trimucronatus*) and mesic tundra habitats. All habitats described here refer to the summer habitat use of the respective species. Further details on vegetation within these habitat types, as well as herbivore fauna in the different study areas are described in Appendix 1, and have been published for Finnmark by (37, 63-65); for Nenetsky by (66) and for Bylot Island by (67, 68). In Table 1, we summarize the populations, years, habitats, and types of analyses for which samples were collected in each study area.

2.2. Population census data and sample collection

In Finnmark and Nenetsky, rodents were trapped as part of monitoring program using the small quadrate-method based on snap-trapping with 12 traps per quadrate over two consecutive nights (69). For each rodent species, we calculated a density index of rodents trapped per 100 trap nights per quadrate (no. rodents/24*100). We used a subset of the trapped rodents for DNA metabarcoding (n = 318 exclusively from Finnmark) and stable isotope analyses (n = 123 from Finnmark, n = 37 from Nenetsky) as described below. Further details on the trapping have been published for meadow habitat in Finnmark (64) and the spatial and temporal distribution of the sampling quadrates are described in Appendix 1.

On Bylot Island, rodents were trapped using snap-trapping and mark-recapture live-trapping (details given in Appendix 1, data published in (70)). A subset of the snap-trapped individuals was used for stable isotope samples (n = 26), in addition to individuals found dead during live-trapping (n = 36). To assess population density, we used estimates obtained through the mark-recapture trapping, which are likely to better reflect actual lemming densities than snap-trapping indices.

2.3. DNA metabarcoding data

Stomach contents of 53 *L. lemmus*, 111 *M. oeconomus*$_{(\text{Finnmark})}$, and 154 *M. rufocanus* from Finnmark study area, collected between 2007 and 2011, were analyzed for seed plant content using DNA metabarcoding. The method is based on first amplifying seed plant DNA using the *g-h* primer pair which targets the P6-loop of the plastid *trn*L (UAA) intron and thereafter high-throughput sequencing the amplified DNA (41, 71). Laboratory analyses of the samples were done in three different batches, but we combined all raw sequencing data prior to sequence annotation to ensure that the data were comparable. The sequences were assigned to plant taxa by comparison with (i) the arctic *trn*L taxonomic reference library (72) (ii) a north boreal *trn*L taxonomic reference library constructed by sequencing 1,332 plant samples

188 representing 835 species (73), and (iii) GenBank, using the program ecoTag. Further details
189 of the bioinformatics analyses are given in Appendix 1. The resulting dataset consisted of a
190 count of sequence reads per taxon per individual rodent. We transformed count data into
191 proportions of plant taxa per individual stomach content to allow for inter-individual
192 comparison. We grouped plant taxa to family level, in order to be able to include most of the
193 data into our analyses (33% of unique sequence reads were annotated to species, 33% to
194 genus, and 30% to family level, respectively). Even though the primer pair *g-h* primarily
195 targets seed plants (Angiosperms and Gymnosperms), some ferns, horsetails and mosses were
196 also identified. We included these into the analyses as groups "mosses" and "ferns and allies".
197 A substantial part of the diet of *L. lemmus* is composed of mosses, but this component of its
198 diet consists rather uniformly of the genus *Dicranum* (74). We could therefore assume that
199 most variation in the species diet occurs within the seed plant component and hence did not
200 include a more comprehensive analysis of mosses in this study.
201
202 ***2.4. Stable isotope samples***
203 Samples of small rodent muscles for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses
204 (hereafter, SIA) were collected on Bylot Island (2008 and 2010), Finnmark (2007-2008 and
205 2011), and Nenetsky (2007-2008). Details for SIA have been published by (75) and (76),
206 except for minor adjustments described in Appendices 1 and 2. To estimate the variability of
207 plant isotopic ratios between species, habitats and localities, we analyzed samples of 21 plant
208 species (n = 280) collected in 2009 in the Finnmark study area. Details of the plant SIA are
209 described in Appendices 1 and 2.
210
211 ***2.5. Data analysis***
212 We used software R 2.14 for all statistical analyses (77).

213 *2.5.1. Trophic niche based on DNA metabarcoding data*

214 DNA metabarcoding data were available for the three populations of Finnmark (Table 1.) To
215 evaluate the effect of population density on trophic niche width, we used as sample units
216 groups of individuals (hereafter "density groups") that were homogeneous in terms of species,
217 year, season, study site (IF, VJ or KO, Figure 1), and habitat (heath, meadow or wetland). We
218 only considered density groups with a minimum of five individuals. Due to low sample size,
219 we grouped individuals across all habitats for *L. lemmus* (n = 51 individuals in total, 28
220 included in this analysis as small density groups were excluded [see above]). For each density
221 group, we calculated an index of trophic niche width for the average diet of the group, using
222 the Shannon entropy (equation given in (5), index denoted hereafter as TNW). We used linear
223 regressions to test, for each species separately, whether *population density index* (predictor
224 variable) had an impact on *TNW* (response variable). To calculate population density index
225 for each density group, we first assigned each individual the density index from the small
226 quadrate where it was trapped. We then calculated an average density index for each density
227 group across individual values. We included *habitat* (heath or meadow) as a covariate in the
228 models for *M. rufocanus* and *M. oeconomus*$_{(\text{Finnmark})}$. We checked for model fit to assumptions
229 using diagnostic plots.

230 We further examined the effect of population density and habitat on diet composition,
231 using individuals as sampling units. We used individual *diet proportions* as a multivariate
232 response variable, with *population density index* (i.e. density index value for an individual in
233 the quadrate it was trapped) and *habitat* (i.e. the habitat where an individual was trapped) as
234 the predictor variables of interest. We analyzed these with Principal Component Analysis with
235 respect to Instrumental Variables (PCAIV) on centered proportions of plant families,
236 implemented with pcaiv-function from ade4-package of the software R (78). To reduce the
237 effect of rare observations, we removed individuals that had fed only on one plant family (n =
238 3, 1, and 2 for *M. rufocanus*, *M. oeconomus*$_{(\text{Finnmark})}$ and *L. lemmus*, respectively), as well as

239 plant families observed in only one individual (n = 3, 2, and 6 for *M. rufocanus*, *M.*
240 *oeconomus*$_{(Finnmark)}$ and *L. lemmus*, respectively). We used forward selection with permutation
241 (5,000 replicates) implemented with forward.sel- function of the packfor-package (79), to test
242 whether covariates should be included (*site* (IF, VJ or KO), *season* (summer or autumn), and
243 *year* (2007-2011)). We only retained covariates significant at α=0.05 level, but always kept
244 habitat and density in the analysis.
245 To evaluate the effect of habitat use expansion on trophic niche width, we used as
246 sample units groups of individuals which were homogenous in terms of species, year, season
247 and study site. For each group, we calculated TNW in two ways; $TNW_{(all\ habitats)}$ including all
248 individuals and $TNW_{(primary\ habitat)}$ including only individuals from primary habitat. We then
249 assessed whether $TNW_{(all\ habitats)}$ was significantly larger than $TNW_{(primary\ habitat)}$, using a re-
250 sampling approach. For each group, we drew 100 times a random combination of individuals
251 (with n equaling that of individuals from primary habitat in the respective group), and
252 calculated TNW for these. However, when the number of possible different combinations was
253 smaller than 100, we calculated TNW for all possible combinations. This was the case for the
254 following groups: *M. rufocanus* 2007 summer KO and VJ, 2010 autumn KO; *M. oeconomus*
255 2007 summer KO and 2011 summer KO; *L. lemmus* 2010 autumn IF and 2011 autumn IF.
256 When the observed difference $TNW_{(all\ habitats)} - TNW_{(primary\ habitat)}$ was above the upper 95%
257 confidence interval of the re-sampled difference (i.e. $TNW_{(all\ habitats)} - TNW_{(resampled)}$), we
258 considered that $TNW_{(all\ habitats)}$ was significantly larger than $TNW_{(primary\ habitat)}$.

259 *2.5.2. Isotopic niche*
260 Analyses of isotopic niche covered all five study populations (Table 1). We used the
261 variability of isotopic ratios – a measure of isotopic niche - as a proxy for tracking the
262 changes in the trophic niche (52, 53). For all analyses of rodents' isotopic niche, we measured
263 isotopic niche width (hereafter referred as INW) as the spread of stable isotope ratios in δ-

space (i.e. a two-dimensional space with one axis for $\delta^{13}C$ and one axis for $\delta^{15}N$; see Figure 2 and 3), estimated via the mean distance to centroid (80, 81). We evaluated changes in isotopic niche composition based on differences in centroid locations (81). For each measure, we used groups of individuals as sampling units and tested for the significance of differences between their distance to centroid and centroid locations using permutation tests described by (81), with 10,000 replicates. See supplementary Table S1 for numbers of individuals included in the different analyses.

To evaluate the effect of population density on isotopic niche width, we divided all five rodent populations into groups of "low" and "high" density. We thus used population density as a categorical variable, to be able to compare groups of individuals, as required by methods of assessing isotopic niche width (80, 81). For Finnmark and Nenetsky, we first assigned to each individual a population density index value (i.e. the density index value from the small quadrate where it was trapped). We then assigned individuals with density index values <10 or >=10 to the "low" and "high" groups, respectively. The "low" index value thus corresponds to one or two individuals trapped in a grid during a trapping event (2/24*100 = 8.3), "high" corresponding to three or more individuals (3/24*100 = 12.5). In Table 1, we summarize the years, seasons, sites, and habitats from which individuals of different populations were included in this analysis. On Bylot Island, population densities in wetland habitats (primary habitat for *L. trimucronatus*) differed little between 2008 and 2010 (Figure 4). However, during 2008 population densities were decreasing, and little spillover of *L. trimucronatus* from wetland to mesic habitat occurred (Figure 4). In 2010 population densities were increasing, and *L. trimucronatus* was abundant in mesic habitat, indicating saturation of wetland habitats. We therefore assigned individuals trapped in 2008 into density group "low" and individuals trapped in 2010 into group "high". Within all populations, we assessed difference in INW between "low" and "high" groups by testing for difference in mean

distance to centroid as described above. Furthermore, to evaluate whether a populations' isotopic niche composition was affected by population density, we tested whether centroid locations of "high" and "low" groups differed (see conceptual illustration of these analyses in Figure 2). We analyzed the differences between low and high densities in two ways; using all individuals and individuals trapped from primary habitats only. For *M. oeconomus*$_{(\text{Nenetsky})}$ all individuals were collected from primary habitat and we therefore did only one analysis.

To evaluate the effect of habitat use expansion on isotopic niche width, we calculated populations INW in two ways; including only individuals from the primary habitat, $INW_{(primary\ habitat)}$, and including all individuals irrespective of habitat, $INW_{(all\ habitats)}$. We then tested whether $INW_{(\text{all habitats})}$ was significantly larger than $INW_{(\text{primary habitat})}$. To assess whether habitat had an impact on isotopic niche composition, we compared pairs of habitat-specific groups of individuals in terms of centroid locations. We included in each pairwise comparison a species primary habitat and one of the secondary habitats. When we had data from several secondary habitats, we compared each of these separately against the primary habitat.

We evaluated the role of confounding effects (site, season, and year) for the observed patterns visually, using isotopic bi-plots. Because we found no directional differences between sites or years in Finnmark (see Supplementary Figure S1 in Appendix 2), we included all data in the analyses. However, as we did find some seasonal patterns, we present them together with the results for density and habitat (Figure 3), and take them into account in our interpretation of results.

2.5.3. Population density data and spillover to adjacent habitats

We assessed the effect of population density on habitat for the three populations of the Finnmark study area (Table 1). In these analyses, we included a subset of the sampling quadrates which are situated so that the study design in each study site was balanced including an equal number of heath and meadow quadrates (until 2008, numbers of quadrates per

habitat were 12 in KO, 13 in VJ and 12 in IF, while from 2009 on they were 10[KO], 9[VJ] and 9[IF]). These quadrates were spatially arranged as pairs, each pair including a quadrate from each habitat. In these analyses, we used pairs of quadrates as sampling units and analyzed for each species separately whether an increase of the *number of individuals trapped in primary habitat* (predictor variable) was related to an increase of the *number of individuals trapped in secondary habitat* (response variable). We run Poisson regressions, implemented with lmer-function of the R-package lme4 (82), including *year* (2007 to 2011), *season* (summer or autumn), *site* (KO, VJ and IF) and *quadrate pair identity* (37 levels) in the models as random variables. We checked model fit to assumptions using diagnostic plots.

3. Results

3.1. Density and trophic niche width (TNW and INW)

We found little indication that trophic niche width of small rodents increased with population density. TNW (analysed for the three Finnmark populations, Table 1) had no significant correlation with population density index in any of the tested populations, although *M. oeconomus* $_{(\text{Finnmark})}$ had a weak increasing trend in its primary habitat (Figure 5, Table 2). INW (analysed for all populations, Table 1), based on mean distance of individuals to centroid, increased significantly with population density only for *L. lemmus*, when individuals from either all habitats or the primary habitat only were included (Figure 3). When we included only individuals from primary habitat, *L. trimucronatus* also showed an increase of INW with density. However, we also found an opposite effect of density on INW in *M. oeconomus* $_{(\text{Finnmark})}$ when individuals from all habitats were included, but not when individuals from only primary habitat were included (Figure 3, Appendix 2; Supplementary Table S2).

3.2. Density and trophic niche composition

Based on DNA metabarcoding data, density had no significant effect on trophic niche composition of any of the studied species (populations included in the analyses are in Table 1, results in Figure 6, Appendix 2; Supplementary Tables S3 and S4). Using stable isotope data, we found species-specific patterns of the effects of density on isotopic niche composition (populations included in the analyses are in Table 1, results in Figure 3, Appendix 2; Supplementary Table S2). Centroid locations differed between low-density and high-density groups for all populations but *L. lemmus* (Figure 3, Appendix 2; Supplementary Table S2). However, for *M. oeconomus*$_{(\text{Finnmark})}$ the pattern disappeared when only individuals from primary habitat were considered. In addition, the density-related patterns could not be confidently distinguished from those caused by season in *M. rufocanus* and *L. trimucronatus* (Figure 3). Data for these populations tended to be collected during different seasons in high and low population densities, and the variation of the individual stable isotope ratios due to density was correlated with the season (Figure 3).

3.3. Density and habitat use expansion

Number of individuals trapped in secondary habitat increased with number of individuals trapped in primary habitat for all three species tested (i.e. all species from Finnmark, Table 1), (Table 3), indicating density-driven spillover from primary to secondary habitats.

3.4. Habitat and trophic niche composition

Based on DNA metabarcoding data, habitat had an impact on trophic niche composition (populations included in the analyses are in Table 1, results in Figure 6, Appendix 2; see also Supplementary Tables S3 and S4). Predictor variables along the first PCAIV-axis predicted 20%, 26%, and 22% of the variation in our data for *M. rufocanus*, *M. oeconomus*$_{(\text{Finnmark})}$, and *L. lemmus*, respectively (Figure 6, Appendix 2; Supplementary Tables S3 and S4). Variables

362 found significant by forward selection were habitat and site (IF differed from VJ but not from
363 KO) for *M. rufocanus*, habitat and year for *M. oeconomus*$_{(Finnmark)}$, and site (IF differed from
364 KO but not from VJ) for *L. lemmus* (Appendix 2; Supplementary Table S4). Habitat was still
365 the most influential predictor explaining the first PCAIV axis for all three species (Figure 6,
366 Appendix 2; Supplementary Table S4), suggesting that for *L. lemmus* the effect of habitat was
367 not detected in forward selection due to low sample size (n = 35, 11 and 5 for heath, meadow
368 and wetland habitats, respectively). Diets reflected the abundance relationships of plant
369 families within the different habitats (described in detail in (37)). For both vole species,
370 ericoid shrubs were associated with heath habitat, whereas forb families, especially
371 Polygonaceae and Ranunculaceae, were associated with meadow habitat. For lemmings,
372 grasses (Poaceae) were associated with heath habitat whereas sedges (Cyperaceae) were
373 associated with wetland and meadow habitats.
374 Based on stable isotope data, i.e. differences of centroid locations, habitat had an
375 impact on isotopic niche for *M. rufocanus* and *M. oeconomus*$_{(Finnmark)}$. This was indicated by
376 the significant difference of centroid location between wetland habitat and primary habitat of
377 the respective species (Figure 3, Appendix 2; Supplementary Table S2). Differences between
378 heath and meadow observed using DNA metabarcoding were not found in the stable isotope
379 data, indicating that the difference in diets between heath and meadow habitats was smaller
380 than between these habitats and the wetland habitat (populations included in analyses are
381 given in Table 1).

382 ***3.5. Habitat use expansion and trophic niche width***

383 Patterns in the effect of habitat use expansion on trophic niche width differed among methods.
384 Based on DNA metabarcoding data, $TNW_{(all\ habitats)}$ was higher than $TNW_{(primary\ habitat)}$ in all but
385 two of the 17 groups tested (Table 4). For all of these groups, the observed difference was
386 larger than the difference between $TNW_{(all\ habitats)}$ and $TNW_{(resampled)}$ (Table 4), indicating a

387 significant increase of TNW with habitat use heterogeneity. On the contrary, stable isotope
388 data showed no similar trends, as we found no difference between $INW_{(all\ habitats)}$ and
389 $INW_{(primary\ habitat)}$ based on mean distance to centroid (populations included in analyses are
390 given in Table 1, results in Figure 3, Appendix 2; Supplementary Table S2).

391 **4. Discussion**

392 ***4.1. Population density and small rodent trophic niche***

393 We found that habitat use was an important determinant of trophic niche at short time scales,
394 based on the DNA metabarcoding data. Habitat was an important determinant of an
395 individual's diet (supporting H3), and heterogeneity in habitat use consequently increased
396 populations' trophic niche width (supporting H4). Furthermore, we observed density-driven
397 spillover from primary to secondary habitats (supporting H2) for all three species in the
398 Finnmark study area. Spillover to adjacent habitats has frequently been related to high
399 population densities in small rodents (13, 32-34). Several driving forces have been suggested
400 for such density-driven increase of population habitat niche width, most prominently resource
401 competition and social competition (35, 83-86). While we cannot determine the cause of the
402 spillover in our study system, we argue that it is unlikely to be caused by competition for
403 food. In the primary habitat, we found no indication for an effect of density on trophic niche
404 width in most populations, except for the two lemming populations over long time scales, as
405 indicated by stable isotope data (H1 being supported only for these populations). Thus,
406 population density did not have a strong impact on diet diversity in the studied small rodent
407 populations. High population density of small rodents seems hence to induce an increase of
408 habitat niche width before competition for food reaches levels that impact population trophic
409 niche width.

Our results imply that habitat-specific food availability is one of the most important determinants of small rodent trophic niche composition. For example, *M. oeconomus*$_{(\text{Finnmark})}$ in the meadow habitats of Finnmark study area select for forbs and willows (37). Availability of these plant groups is lower in the heaths than in the meadows, and their taxonomic composition differs (37). Subsequently, *M. oeconomus*$_{(\text{Finnmark})}$ need to adjust their feeding habits in different habitats, which is illustrated by our results. The effect of habitat niche expansion on trophic niche width is, however, likely to differ between small rodent populations based on the similarity of plant species pools between habitats. For example, the most important vascular plant food item of *L. lemmus* in the Finnmark study area is the grass *Avenella flexuosa* (74). This grass species is abundant in both heath and meadow habitats (87), and thus *L. lemmus* probably faces comparatively little need to adjust its diet when moving between these habitats. This illustrates that some herbivore species may maintain their preferred diet in another habitat simply because the preferred food items are available there as well. Furthermore, food availability can be strongly reduced by predation risk, which again is modified by the availability of sheltering vegetation (88). Hence, the extent to which a populations' habitat use modifies its trophic niche width most likely varies between species based on both their food preferences as well as habitat-specific availability of food and shelter from predators.

It has been suggested that certain plant species would be included in small rodent diets exclusively at high population densities, causing such a reduction of diet quality that the population dynamics are affected (23-25). Our results indicate that this is unlikely to be the case, at least for the population densities observed in this study. We found species-specific patterns in the direct effects of density on population trophic niche width within the primary habitat, and little unambiguous evidence for a change in population trophic niche composition due to density. On the other hand, food availability is an important determinant of small

435 rodent diets, both among habitats, as indicated by our results, and within habitats (37). Any
436 change in an individual's diet, which is caused by population density, is therefore likely to
437 depend on what is available for different individuals in terms of food quality and quantity.
438 These, in turn, can be modified by various local factors, such as predation risk and shelter
439 availability. Individuals can, therefore, be expected to differ in terms of how population
440 density impacts their diet. It thus seems unlikely that the quality of a single food item,
441 included in the diet of a rodent population only at high population densities, would have such
442 impacts on reproduction or mortality that the population dynamics would be affected.
443 Our results differ between species in many aspects, indicating that different herbivore
444 species, even within a relatively homogeneous guild, may show different trophic responses to
445 increased density. One explanation of such differences is that the impact of competition on
446 herbivore diet is likely influenced by the degree of specialization of the herbivores. For
447 example, lemmings have in general more specialized feeding habits than voles (16, 37, 74).
448 Consequently, they may experience exploitation competition, causing a diversification of diet,
449 at population densities which would not impact the trophic niche width of voles. Herbivore
450 species trophic niche width response to high densities may also be partly determined by the
451 impact of herbivores on vegetation. For example, the results of (9, 11) suggest that intensive
452 grazing by ungulates would reduce plant species richness, thus leading to a decreased trophic
453 niche width. High population densities of ungulates may persist over long time periods and
454 indeed often have drastic effects on vegetation diversity (89-91). On the other hand, the
455 period of intense grazing by cyclic small rodent populations lasts only a year or two, and
456 impact on vegetation is sometimes limited (e.g. Bylot Island; (92)). Small rodents may thus
457 interact with vegetation diversity in a different manner than larger herbivores. Our results
458 underline that the effects of competition on the trophic niche of herbivore population can be
459 both direct and indirect, and depend greatly on the ecology of the species in question. For

instance the degree of diet specialization, interplay between high population densities and vegetation diversity as well as dispersal to adjacent habitats may modify either the direct or indirect effects of competition. This urges further studies on the effects of competition on herbivore trophic niches to consider, in addition to direct effects, both indirect effects and interactions between herbivores and their food plants.

4.2. Use of stable isotopes and DNA metabarcoding in herbivore diet studies

The use of DNA metabarcoding and SIA in diet studies has recently been discussed in detail in publications focusing on one of the methods (47, 49, 93). We focus here on the combination of these two methods, illustrating how they may be used in a complementary manner in diet studies.

We obtained several method-specific results. For example, we found clear differences in trophic niche composition between heath and meadow habitats for the vole species using DNA metabarcoding. SIA, on the other hand, indicated that diets of voles differed between their respective primary habitat and wetland habitat, but not between heath and meadow habitats. These discrepancies illustrate the importance of different temporal resolution between these two types of data. While DNA metabarcoding of stomach contents captures the last meal, stable isotopes can incorporate information over a much longer time-scale (94, 95). Although no data on muscle turnover rates of our study species exist, based on data from other rodent species (95, 96) we can assume that the present isotopic ratios reflect average diets during the last month. Because plant species identity was the main source of plant isotopic variation and habitat was a strong predictor of short-term diets, we would have expected habitat-specific differences in small rodent stable isotope ratios. As this was not the case, the sampled small rodents were probably not exclusively feeding in the habitat where they were captured during the last month. Some of the sampled individuals may for example have migrated from primary to secondary habitats or included several habitats in their home-

485 ranges. While the sampling quadrates covering heath and meadow habitats were situated in
486 each other's vicinity, the wetland habitat quadrates were spatially more segregated. Thus,
487 food availability in the area where an individual was moving the month prior to trapping
488 differed probably less between heath and meadow than between wetland and the other
489 habitats. This underlines the importance of considering processes at appropriate temporal and
490 spatial scales, such as the effect of habitat-specific food availability over the short-term and
491 residency time within habitat over the longer term.

492 In our study, DNA metabarcoding could describe the composition of current diets and
493 their spatial variability. However, the difference in food availability between habitats is
494 probably greater at plant species level than at the family level. Hence, the actual effect of
495 habitat-specific food availability on diets is probably larger than what we observed in our
496 family level analyses. Future studies may therefore benefit from new developments of DNA
497 metabarcoding offering higher species level resolution (43). On the other hand, stable isotope
498 data illustrated that spatial variability of trophic niche does not necessarily persist over time.
499 In principle, stable isotope ratios of different tissues alone could give indication of the spatial
500 and temporal variation in diets (6, 54). However, herbivore diet composition cannot, in most
501 cases, be inferred from their stable isotope ratios due to the large number of potential food
502 items and the overlap between their stable isotope ratios (e.g. present study). However, a
503 combination of SIA and DNA metabarcoding may elucidate herbivore feeding ecology when
504 both current diet composition and temporal variability are of interest. For example, when parts
505 of the life-cycle of the herbivore in question are cryptic or otherwise inaccessible, stable
506 isotope samples from a tissue with slow turnover can provide a way to study past diets. For
507 small rodents, such an application could be especially of interest in studying feeding habits
508 during winter, which is a critical season in terms of food limitation, but difficult to study
509 otherwise. However, a comprehensive understanding of the temporal variation in underlying

plant stable isotope ratios would be required to properly exploit the possibilities of stable isotopes in describing temporal changes of herbivore diets.

The approach outlined above to combine DNA metabarcoding and SIA is discussed with a focus on diet studies of terrestrial herbivores, while different approaches may come into question for different types of consumers. For example, DNA metabarcoding of predator diets is often more difficult than that of herbivores, due to the inherent problem of prey DNA getting swamped by the predators DNA (47). For SIA the situation is the opposite, i.e. predator diet composition is often easier to assess than that of herbivores, due to a lower number of food items with more distinct stable isotope ratios (97). On the other hand, depending on the question very different analytic approaches could be used, as is illustrated by (58), who evaluated different carbon sources of a river ecosystem rather than attempting to quantify consumer food sources. Hence, the suitability of a combination of DNA metabarcoding and SIA should be carefully assessed based on the specific study systems and questions.

Conclusions

Our results indicate that for arctic small rodents, the impact of high population density is mostly manifested as spillover to adjacent habitats before the competition for food in primary habitat is strong enough to have an impact on population trophic niche width or composition. Small rodent diets reflect food availability, and hence a density-driven increase in population habitat niche width leads to an increase in population trophic niche width as well. However, the effects of competition on herbivore trophic niche can differ between species or guilds of herbivores, while the roles of different potential drivers, such as temporal persistence of intensive grazing and degree of diet specialization remain unknown. To evaluate these drivers, a combination of DNA metabarcoding and SIA can be a useful approach, especially when both current diet composition and temporal changes are in the focus. However, this

methodological approach should be used with caution and the potential pitfalls assessed thoroughly.

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Conflict of interest

L.G. is one of the co-inventors of a patent concerning g-h primers and the subsequent use of the P6 loop of the chloroplast *trn*L (UAA) intron for plant identification using degraded template DNA. These patents only restrict commercial applications and have no impact on the use of this locus by academic researchers.

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Table 1. Summary of populations, sample types, analyses and sample sizes included in this study.

Population	DNA	SIA	H	Analyses	Comparisons	n group	n ind	Data included
Lemmus lemmus	Yes	Yes	1	DNAniche width~density	Individuals from same year/season/site	4	28	2007, 2010, 2011; H, M, W; September; VJ, KO
Finnmark, Norway			4	DNAniche width ~habitat use	Individuals from same year/season/site/habitat	2	34	2007, H, M, W; September; VJ, KO
			1,3	DNAniche composition	-		51	2007-2011, H, M; July, September, IF, VJ, KO
			1,3,4	All SIAniche analyses	Density class groups/ habitat groups	2/3	28 (16)	2007; H, M, W, September; VJ, KO
Microtus oeconomus	Yes	Yes	1	DNAniche width ~density	Individuals from same year/season/site/habitat	7	94	2007, 2011; H, M; July, September; VJ, KO
Finnmark, Norway			4	DNAniche width ~habitat use	Individuals from same year/season/site/habitat	3	61	2007, 2011; H, M; July, September; VJ, KO
			1,3	DNA niche composition	-		111	2007-2011, H, M; July, September; IF, VJ, KO
			1,3,4	SIAniche all analyses	Density class groups / habitat groups	2/3	36 (18)	2007-2011, H,M, W; June, July, September; IF, VJ, KO
Myodes rufocanus	Yes	Yes	1	DNAniche width ~density	Individuals from same year/season/site/habitat	11	128	2007-2011, H, M; July, September; IF, VJ, KO
Finnmark, Norway			4	DNAniche width ~habitat use	Individuals from same year/season/site/habitat	8	110	2007-2011, H, M; July, September; IF, VJ, KO
			1,3	DNAniche composition	-		153	2007-2011, H, M; July, September; IF, VJ, KO
			1,3,4	All SIAniche analyses	Density class groups/ habitat groups	2/3	59 (31)	2007-2011, H, M; June, July, September; IF, VJ, KO
Lemmus trimucronatus	No	Yes	1,2	SIAniche all analyses	Years / habitat groups	2/2	62 (36)	2008, 2010; mesic, wet; June, July, August
Bylot Island								
Canada								
Microtus oeconomus	No	Yes	1	SIAniche ~density	Density class groups	2	37 (37)	2007, 2008; meadow; July, August
Nenetsky,Russia								

Subscript Table 1: Column “DNA”= DNA metabarcoding data; column “SIA”= stable isotope analyses; column “H”= number of hypotheses presented in the introduction (H1-H4); column “Analyses” = analyses (“DNAniche” = analyses using DNA-data, “SIAniche” = analyses using stable isotope data); column “n group” = number of sampling unit groups (for isotopic niche, first number is for density class groups, second number for habitat groups); column “n ind” = number of individuals (for isotopic niche, first number is all individuals, second number individuals from primary habitats); column “Data included” = samples included (years; habitats (for Finnmark, H=heath, M=meadow and W=wetland); months; sites (for Finnmark, IF= Ifjord, VJ= Vestre Jakobselv, KO= Komagdalen).

Table 2. Effect of population density index on the total niche width (stomach content data, Finnmark study area, Norway). Parameter estimates based on linear regression. Intercept level for habitat is heath. Predictor variables for which 90% or 85% confidence interval does not cross zero are denoted in bold or italics, respectively.

Species	Predictor	Est.	95 % CI	R^2adjusted
Myodes rufocanus	Intercept	1.35	0.76, 1.94	
(n = 11 groups)	Density	0.02	-0.03, 0.06	
	Habitat (M)	0.26	-0.18, 0.69	-0.0007
Microtus oeconomus	Intercept	1.63	1.00,2.28	
(n = 7 groups)	*Density*	*0.02*	*-0.01, 0.05*	
	Habitat (M)	**-0.47**	**-1.04,0.05**	0.51
Lemmus lemmus	Intercept	1.56	-1.33, 3.26	
(n = 4 groups)	Density	-0.006	-0.12, 0.10	-0.45

Table 3. The effect of population density index in primary habitat on population density index in secondary habitat (Finnmark study area, Norway). Parameter estimates from generalized linear mixed effect model with Poisson-distribution. For all populations, n = 316 trapping quadrate pairs. Response variable (i.e. density in secondary habitat) is given below species name. Estimates for intercept and fixed predictor variable (i.e. density in primary habitat, M denotes meadow and H heath) are shown with standard error, z-value and p-value of the Wald z-test, and for random effects with standard deviation of variance (SD, random effects). Predictor variables which had a significant effect (defined as $p < 0.05$) are denoted in bold.

Species		Estimate (SE)	Z	p	SD
M. rufocanus	Intercept	-2.86 (0.93)	-3.07	0.002	
Meadow	**Density (H)**	**0.13 (0.06)**	**2.03**	**0.04**	
	Quadrate pair				0.92
	Site				1.14
	Year				1.23
	Season				0.30
M. oeconomus	Intercept	-4.27 (1.21)	-3.53	0.0004	
Heath	**Density (M)**	**0.19 (0.05)**	**3.56**	**0.0004**	
	Quadrate pair				0.87
	Site				0.89
	Year				1.98
	Season				0.60
L. lemmus	Intercept	-3.78 (1.46)	-2.59	0.01	
Meadow	**Density (H)**	**0.12 (0.05)**	**2.67**	**0.008**	
	Quadrate pair				0.60
	Site				0.87
	Year				2.67
	Season				0.64

Table 4. Difference of total niche width (TNW) between groups of small rodent individuals from all habitats and primary habitat only, Finnmark study area, Norway. Column "Diff. obs." refers to the observed difference (i.e. $TNW_{(all\ habitats)} - TNW_{(primary\ habitat)}$). Column "Diff. resampled" refers to mean (95% CI) difference between $TNW_{(all\ habitats)}$ and $TNW_{(resampled)}$. Groups for which the observed difference was higher than the upper 95% CI limit of the resampled difference are written in bold.

Species	Year	Season	Site	Diff. obs.	Diff. resampled	N	Prop
Myodes	**2007**	**autumn**	**KO**	**0.12**	**0.005 (-0.002,0.01)**	**26**	**0.92**
rufocanus	2007	summer	KO	0.01	0.004 (-0.0006, 0.01)	23	0.96
	2007	**autumn**	**VJ**	**0.19**	**0.02 (0.007, 0.03)**	**30**	**0.87**
	2007	**summer**	**VJ**	**0.14**	**0.02 (-0.0006, 0.04)**	**12**	**0.83**
	2008	**autumn**	**IF**	**0.47**	**0.13 (0.10, 0.17)**	**13**	**0.46**
	2008	**summer**	**IF**	**0.18**	**0.10 (0.08, 0.12)**	**12**	**0.58**
	2010	autumn	IF	-0.014	0.05 (0.03, 0.07)	13	0.69
	2010	**autumn**	**KO**	**0.43**	**0.32 (0.28,0.37)**	**5**	**0.60**
	2011	**summer**	**IF**	**0.43**	**0.18 (0.16, 0.21)**	**12**	**0.50**
Microtus	**2007**	**autumn**	**KO**	**0.19**	**0.03 (0.02, 0.05)**	**25**	**0.80**
oeconomus	**2007**	**summer**	**KO**	**0.07**	**0.002 (-0.001, 0.01)**	**31**	**0.97**
(Finnamrk)	**2011**	**summer**	**KO**	**0.5**	**0.31 (0.22, 0.35)**	**8**	**0.50**
	2011	**autumn**	**VJ**	**0.24**	**0.09 (0.08, 0.11)**	**22**	**0.50**
Lemmus	**2007**	**autumn**	**KO**	**0.18**	**0.04 (0.03, 0.06)**	**20**	**0.75**
lemmus	**2007**	**autumn**	**VJ**	**0.37**	**0.04 (0.02, 0.07)**	**19**	**0.68**
	2010	**autumn**	**IF**	**0.44**	**0.15 (-0.01, 0.41)**	**5**	**0.60**
	2011	**autumn**	**IF**	**0.63**	**0.16 (-0.01, 0.45)**	**5**	**0.60**

Subscript Table 4: Column "N"= number of individuals for $TNW_{(all\ habitats);}$ column "prop" = proportion of N consisting of individuals for primary habitat.

Figure captions

Figure 1. Map of the study areas. Small map presents the study sites within Finnmark study area, Norway (IF = Ifjordfjellet, VJ = Vestre Jakobselv, KO = Komagdalen). Color codes (A to E) represent vegetation zones of the Arctic, according to (98): A-Polar desert; B-High-Arctic tundra; C-Typical Arctic tundra; D-Low Arctic tundra; E: Arctic Shrub-tundra.

Figure 2. Conceptual representation of isotopic niche width (INW) and composition, as used in the present study.

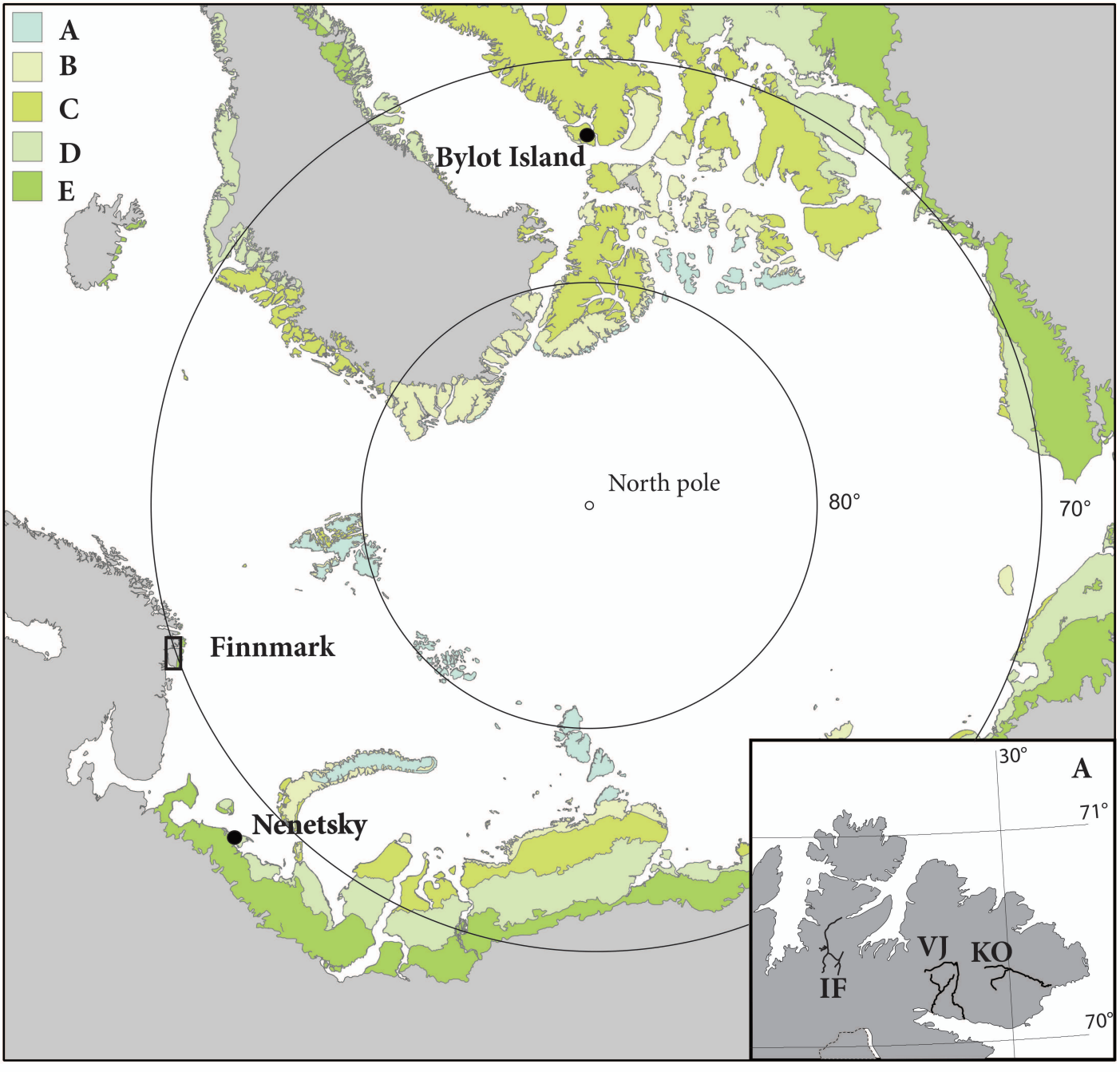
Figure 3. Stable isotope ratios of carbon and nitrogen for individuals from five populations of small rodents, data from all the three study areas. Populations are shown on different rows. Columns show analyses within populations; "density all" = population density groups; "density primary" = population density groups including individuals from primary habitats only; "habitat" and "season". For the test of 1) difference in isotopic niche composition between groups we show centroid coordinates of each group (larger points) and p-values for significant differences between these (below the legend). For the test of 2) isotopic niche width we give 90% confidence ellipses, bars in lower right corner showing mean (with SE) distance to centroid (at the scale of the y-axis of the respective plot) and p-values for significantly higher distances to centroid above the bar in question. In the habitat analyses the category "combined" shows all habitats. We tested whether isotopic niche width of combined habitats differed from that of primary habitat. Letters indicate which groups were compared (H = heath, M = meadow, W = wetland). More details are given in Appendix 2; Supplementary Table S2. Empty plots indicate lack of data.

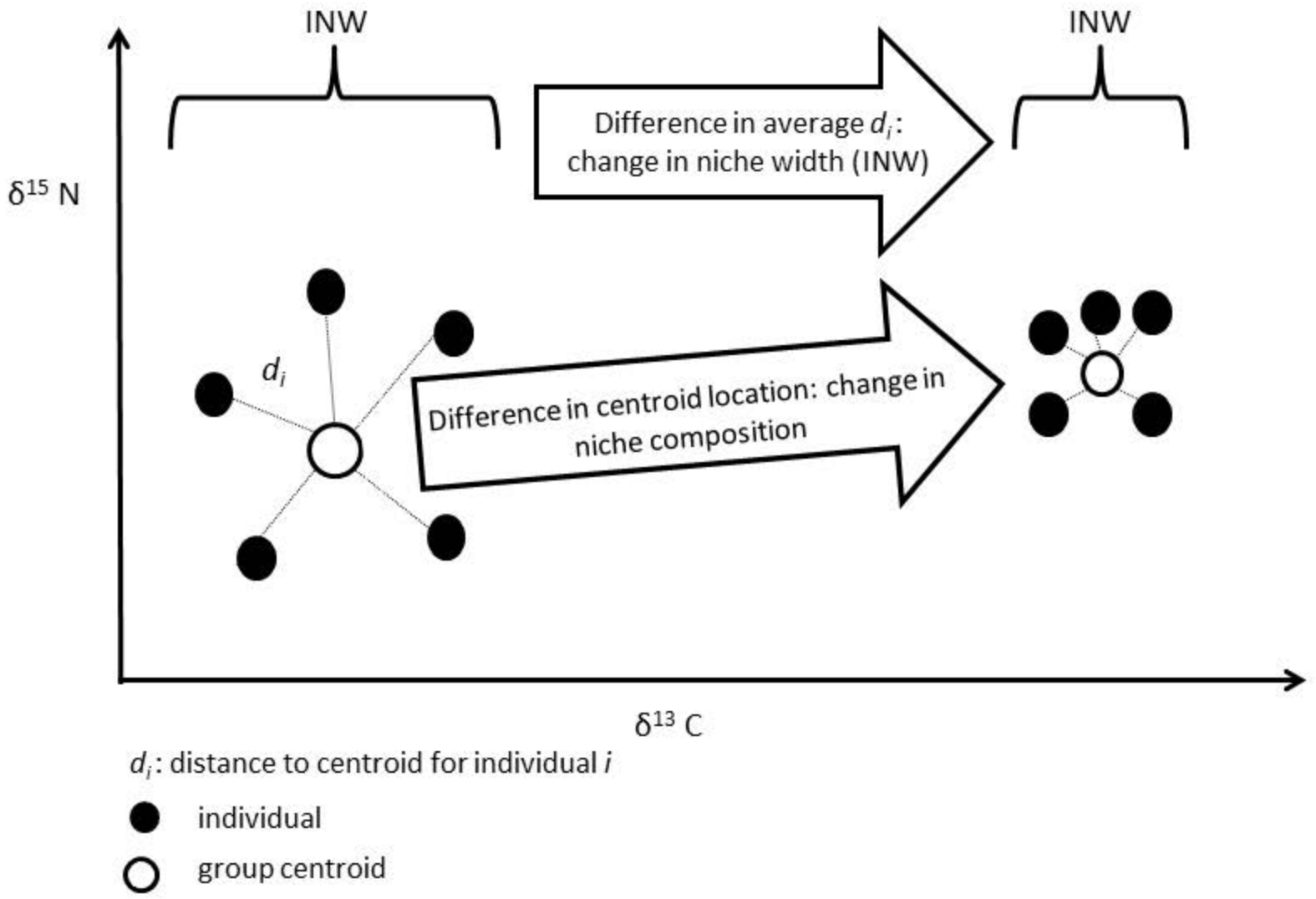
Figure 4. Population dynamics of small rodents in the study areas during years of sampling. For Finnmark population density index (individuals / 100 trap-nights) is estimated as the mean across heath and meadow quadrates. For Nenetsky only data from meadow-habitat are included. For Finnmark and Nenetsky J=July, A=August, S=September, for Bylot Island Jn=June, Jl=July (Jl1 early July, Jl2 late July), A=August. Data from Finnmark is separated between study sites ; KO= Komagdalen, VJ = Vestre Jakobselv, IF = Ifjordfjellet.

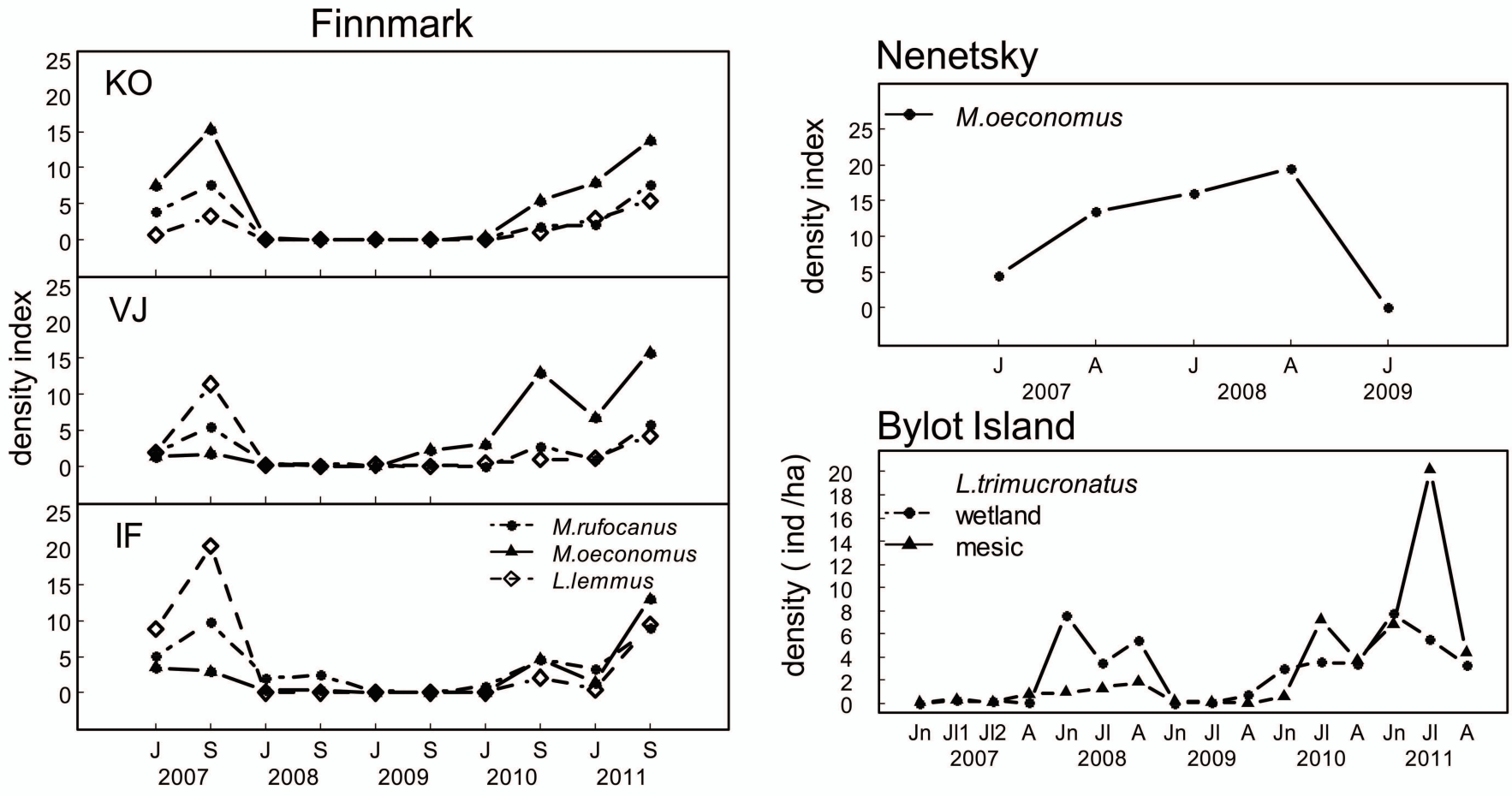
Figure 5. Total niche width (TNW) and population density index (individuals / 100 trap nights) for the three small rodent populations in the Finnmark study area.

Figure 6. Population density (den) and habitat (hab) effects on trophic niche composition (i.e. stomach content proportions based on DNA metabarcoding data) for the three small rodent populations in the Finnmark study area. Upper panels show unconstrained PCA plots, middle panels PCA constrained with predictor variables which are shown in lower panels (PCAIV & PCAIV loadings). The degree of similarity between PCA and PCAIV plots reflects the extent to which predictor variables can account for the structure in diet variation. If a plant family (in PCAIV plot) is in the vicinity of a predictor variable vector (PCAIV loading plot), they are positively correlated. X-axes represent 1st PCA /PCAIV axis, y-axes 2nd PCA/PCAIV axis. Inset plots show eigenvalues for each analysis, 1st bar to the left representing 1st PCA/PCAIV axis (lengths of 1st axes given in subscript below the figure). Plant family names have been abbreviated to three first letters (see subscript below the figure; open font is used to clarify overlapping names), as is done for predictor variables (PCAIV loadings plots; habM = meadow, habW = wetland, siteKO = Komagdalen, siteVJ = Vestre Jakobselv). The grey box in the middle represents all remaining plant families. PCAIV results are given in Supplementary Tables S3 and S4. For example, variability in *M. oeconomus* diet was for a large part accounted by variability in proportion of Polygonaceae (uppermost panel, first PCA axis). This variation was explained by difference between heath and meadow habitats; first PCAIV axis shows Polygonaceae separately from other families (middle panel), correlating well with the position of meadow habitat predictor variable along first PCAIV axis (lowest panel).

Subscript figure 6: Eigenvalue of 1st PCA/PCAIV axis, upper row left to right; 0.14, 0.13, 0.15; middle row all plots; 0.03. Ast=Asteraceae, Bet=Betulaceae, Cor=Cornaceae, Cyp=Cyperaceae, Eri=Ericaceae, fer= ferns and allies, Ger = Geraniaceae, Jun=Juncaceae, Pol=Polygonaceae, Poa=Poaceae, Ran=Ranunculaceae, Ros=Rosaceae


A
B
C
D
E
Bylot Island
North pole
80°
70°
Finnmark
Nenetsky
30°
A
71°
VJ
KO
IF
70°


INW
INW
Difference in average d_i: change in niche width (INW)
δ^{15} N
d_i
Difference in centroid location: change in niche composition
δ^{13} C
d_i: distance to centroid for individual i
individual
group centroid


Finnmark
KO
VJ
IF
density index
M.rufocanus
M.oeconomus
L.lemmus
J S J S J S J S J S
2007 2008 2009 2010 2011
Nenetsky
M.oeconomus
density index
J A J A J
2007 2008 2009
Bylot Island
L.trimucronatus
wetland
mesic
density (ind /ha)
Jn Jl1 Jl2 A Jn Jl A Jn Jl A Jn Jl A Jn Jl A
2007 2008 2009 2010 2011

density all | density primary | habitat | season

M.rufocanus — high / low — 0.01 | 0.03 | heath / meadow / wetland / combined — H-W 0.01 | spring / summer / autumn

M.oeconomus (F) — high / low — <0.01 | 0.01 | heath / meadow / wetland / combined — M-W <0.01 | spring / summer / autumn

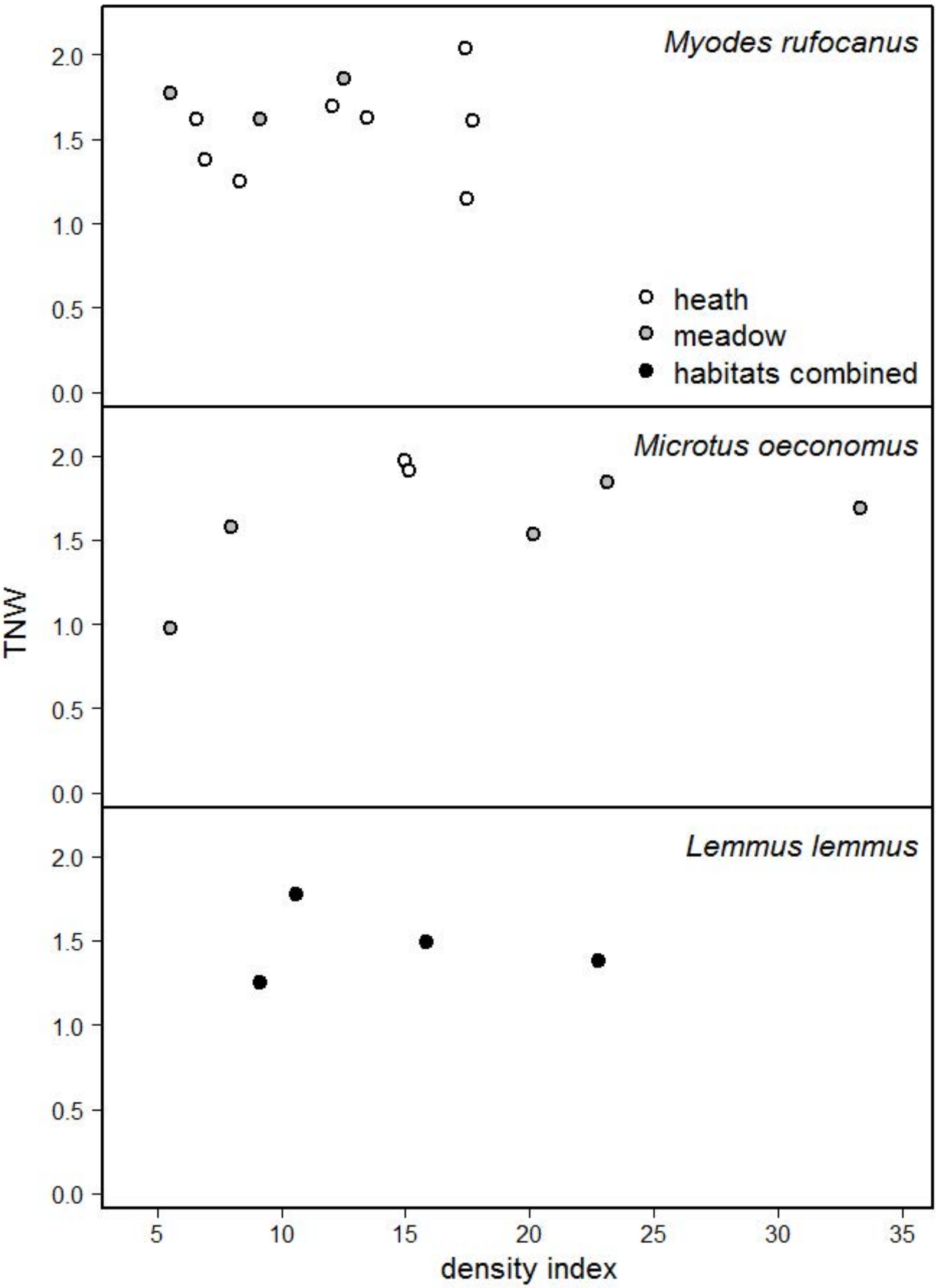
L.lemmus — high / low — 0.005 | 0.03 | heath / meadow / wetland / combined

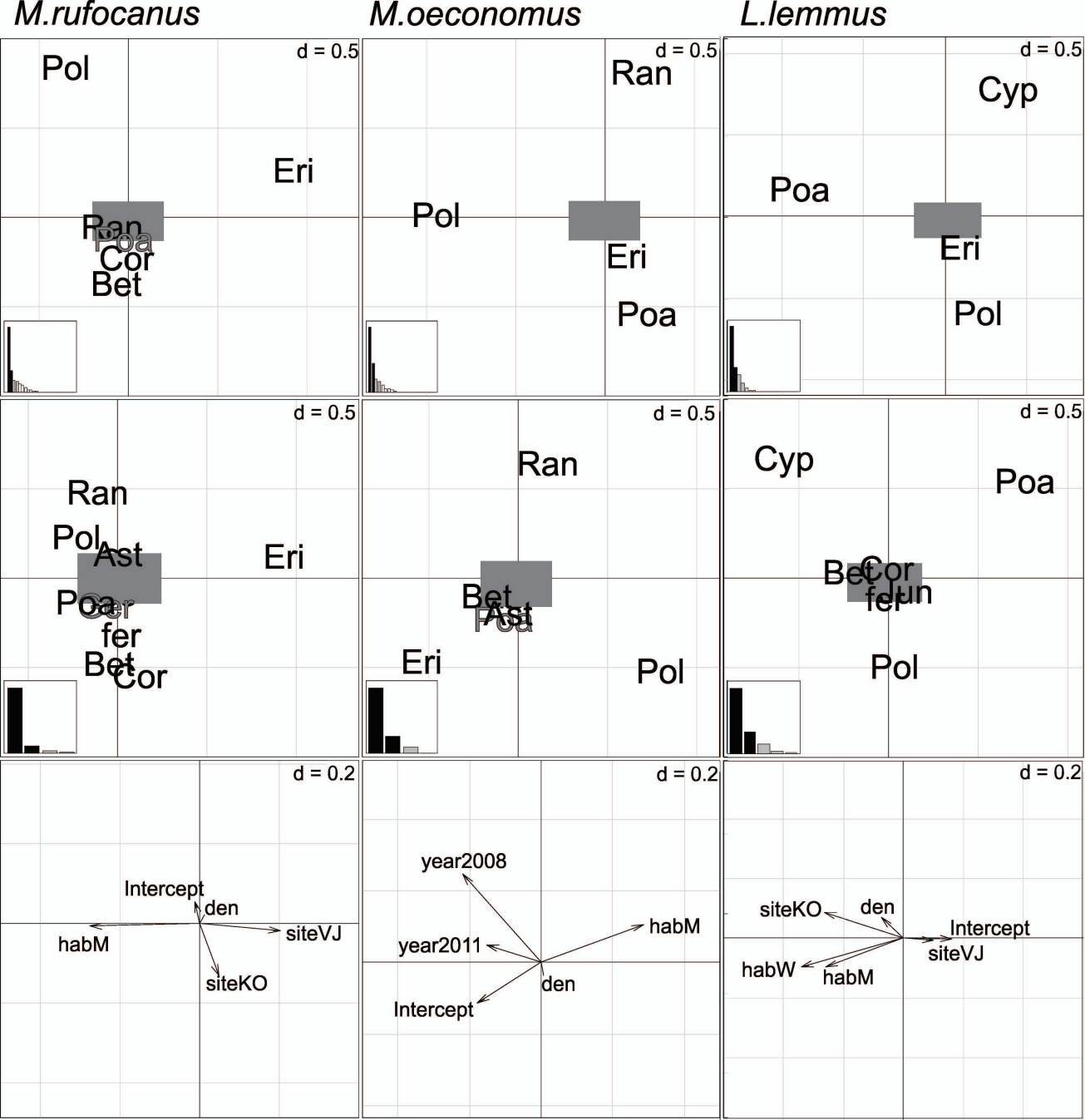
M.oeconomus (N) — high / low — 0.01 | July / August

L.trimucronatus — high / low — <0.01 | <0.01 | wet / mesic / combined — 0.04 | June / July / August

δ^{15} N

δ^{13} C


Myodes rufocanus
heath
meadow
habitats combined
Microtus oeconomus
Lemmus lemmus
TNW
density index
0.0
0.5
1.0
1.5
2.0
5
10
15
20
25
30
35


M.rufocanus
M.oeconomus
L.lemmus
d = 0.5
Pol
Eri
Ran
Poa
Cor
Bet
d = 0.5
Ran
Pol
Eri
Poa
d = 0.5
Cyp
Poa
Eri
Pol
d = 0.5
Ran
Pol
Ast
Eri
Poa
fer
Bet
Cor
d = 0.5
Ran
Bet
Ast
Eri
Pol
d = 0.5
Cyp
Poa
Bet
Cor
fer
Pol
d = 0.2
Intercept
den
habM
siteVJ
siteKO
d = 0.2
year2008
habM
year2011
den
Intercept
d = 0.2
siteKO
den
Intercept
siteVJ
habW
habM