Shedding new light on the diet of Norwegian lemmings: DNA metabarcoding of stomach content

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Abstract

Lemmings are key herbivores in many arctic food webs and their population dynamics have major impacts on the functioning of tundra systems. However, current knowledge of lemming diet is limited, hampering evaluation of lemming-vegetation interactions. This lack of knowledge is mainly due to methodological challenges, as previously used microhistological methods result in large proportions of poorly resolved plant taxa. We analysed diets of Norwegian lemmings (*Lemmus lemmus*) in three different habitats using a new method, DNA metabarcoding of stomach contents. To achieve detailed information on ingested vascular plants, bryophytes and fungi, we amplified short fragments of chloroplast DNA (for plants; P6 loop of the *trn*L intron) and nuclear ribosomal DNA (for fungi; ITS1 – region). Our results revealed that lemming diets were dominated by grasses, mainly *Avenella flexuosa,* and mosses, mainly *Dicranum* spp., but that a variety of other food items were also eaten. Vascular plant composition of the diets differed between heath, meadow and wetland habitats, whereas bryophyte composition did not. Also a variety of fungal taxa were retrieved, but as most of the identified taxa belong to micromycetes, they were unlikely to be consumed as food. The role of fungi in the diet of lemmings remains to be investigated. We suggest that there may be substantial variation between habitats and regions in lemming diet.

Keywords: Small rodents, *Lemmus lemmus*, tundra, herbivore, *trn*L approach, fungi

Introduction

In most tundra ecosystems, lemmings function as the main trophic link between vegetation and predators (Krebs et al. 2003; Ims and Fuglei 2005; Krebs 2011). Hence, their high amplitude population density cycles often have a major impact on tundra food webs (Moen et al. 1993; Gauthier et al. 2004; Henden et al. 2008). To correctly evaluate the effect of lemmings on vegetation - and vice versa - it is crucial to identify what they feed on in the wild, especially since lemming cycles may be driven by plant-herbivore interactions (Turchin et al. 2000; Ekerholm et al. 2001; Oksanen et al. 2008). Knowledge of lemming diet, especially for the Norwegian lemming (*Lemmus lemmus*), in the wild is, however, scarce (Tast 1991; Batzli 1993; Saetnan et al. 2009; Krebs 2011). Therefore, studies of vegetation-lemming interactions often have to make assumptions based on the sparse data available from other areas or habitats (Andersson and Jonasson 1986; Morris et al. 2000; Olofsson et al. 2004) or use generalizations like "broad diet" (Aunapuu et al. 2008) or "moss eaters" (Turchin et al. 2000). Such a lack of knowledge hampers our understanding of lemming-vegetation interactions, and finally our ability to understand the role of lemmings as a trophic link.

Most of the uncertainty about Norwegian lemming diets arises from the small sample size in studies analyzing stomach contents (but see Koshkina (1961) and Tast (1991)) and the coarse categories used to define diet (but see Saetnan et al. (2009)), precluding the generalization of former observations. Low sample size and coarse classification mainly result from

65 methodological limitations, as stomach content analysis of rodents using microscopy is time-
66 consuming, and often has low taxonomic resolution (Soininen et al. 2009). In addition, the
67 potential role of fungi in affecting the diet quality of small rodents has been emphasized
68 (Saikkonen et al. 1998; Huitu et al. 2008), but their abundance and identity in lemming diets
69 are hardly accessible with microhistological methods. As an alternative, DNA metabarcoding,
70 i.e. DNA barcoding of environmental samples coupled with large scale parallel high-
71 throughput sequencing techniques (as defined by Taberlet et al. (2012)), has lately been
72 successfully used to study herbivore diets (Pegard et al. 2009; Kowalczyk et al. 2011; Raye et
73 al. 2011; Pompanon et al. 2012). This approach consists of amplifying and sequencing a
74 standardized DNA region from feces/stomach content, and subsequently identifying and
75 quantifying the organisms composing the diet by comparing the obtained sequences to a
76 reference database (see review by Valentini et al. (2009)). Compared to traditional methods
77 for herbivore diet analysis, DNA metabarcoding provides finer taxonomic resolution, has the
78 potential to identify more taxa, and analyze a large number of samples in addition to being
79 less likely biased by the observer (Soininen et al. 2009; Valentini et al. 2009)

80

81 We present here the first species level data on the diet of Norwegian lemmings, using DNA
82 metabarcoding. The species is believed to feed largely on mosses during winter and on a
83 wider variety of forbs, graminoids and shrubs in the summer (Kalela et al. 1961; Koshkina
84 1961; Stoddart 1967; Hansson 1969; Tast 1991; Batzli 1993; Saetnan et al. 2009). To further
85 assess the variability of Norwegian lemming diets, we used a DNA metabarcoding approach
86 on stomach contents collected during a population peak in different habitats in a low arctic
87 region of Finnmark, north-eastern Norway. To achieve taxonomically detailed information of

both vascular plants and bryophytes, we used two different primer sets to identify the ingested plants (Taberlet et al. 2007). As the first attempt to evaluate identity of the fungi ingested by Norwegian lemmings, we also analyzed the stomach content using a primer pair developed for DNA metabarcoding of fungi (Epp et al. 2012).

Material and Methods

Study area and samples

All samples were collected in the Varanger Peninsula in the north eastern part of Norway (70-71° N, 28-31° E), in 2007, using snap-trapping (cf. Henden et al. (2011)). The area is classified as low arctic tundra (Walker et al. 2005). During the summer of 2007 Norwegian lemming populations peaked in the area, followed by a population crash during the winter of 2008 (Henden et al. 2011; Ims et al. 2011). The samples were mainly collected in early September (n=39), but to achieve a more balanced sample size between habitats one individual trapped in late June was included in the analyses. Samples were collected from two different river catchment areas, namely Komagdalen and Vestre Jakobselv (n=20 for both areas respectively). In both river catchments, three types of habitats were sampled; (1) alpine low-shrub heaths dominated by *Empetrum nigrum* s. lat., *Vaccinium* spp. and *Betula nana*, (2) meadows dominated by grasses and forbs, with interspersed willow shrubs (*Salix* spp.) and (3) wetlands, dominated by *Carex* spp. and low shrubs (*Salix* spp., *Betula nana*). Most samples were collected from heaths (n=28), whereas sample sizes for meadows and wetlands were lower (n=5 from each habitat, respectively). Two individuals could not be assigned to these habitat categories, and data from these was excluded from the

comparison between habitats. Difference between the two river catchments was not assessed due to low sample size for meadow and wetland habitats. The mean weight of the sampled Norwegian lemmings was 50g (±16 SD, n=22) for females and 50g (±11 SD, n=17, weight lacking for one individual) for males. The sampled Norwegian lemmings contained both adults and juveniles, although age was not determined for all individuals. For females, n=6 adults, 3 juveniles and 14 unknown, for males n=5 adults, 3 juveniles and 8 unknown. Part of the Norwegian lemmings (n=16) were dissected in the field and their stomachs stored in 70% ethanol. The remaining individuals (n=24) were frozen and dissected later at the laboratory. All stomachs were opened in the laboratory and contents were homogenized and dried.

Diet analysis

Stomach contents were analyzed using DNA metabarcoding. Identity and abundance of plants in stomachs was assessed using two universal primer pairs for plants, which both use the P6-loop of the chloroplast trnL (UAA) intron; *g-h* and *c-h* (Taberlet et al. 1991; Taberlet et al. 2007). The *g-h* primer pair gives taxonomically relatively precise results for small rodent diets (Soininen et al. 2009). Its provides, however, results biased towards seed plants. To achieve a complementary picture of all plant taxa in Norwegian lemming diets we also used primer pair *c-h*, which is universal for all plant taxa (bryophytes included). We analyzed presence of fungi using primer pair *ITS-Fungi*, which is developed for DNA metabarcoding approaches and combines primers ITS5 and 5.8S_fungi (White et al. 1990; Epp et al. 2012). One sample per individual was analyzed following the methods for DNA extraction, amplification, quantification and tagging described in detail by Soininen et al. (2009).

133 Sequencing was done by the Génoscope (French National Sequencing Center, EVRY), on a
134 454 GS FLX sequencer (Roche Diagnostics) using Titanium chemistry. Details on retrieving
135 taxonomic units based on raw sequence data are given, for each primer pair separately, in
136 Supplementary Table S1.

137

138 As taxonomic reference libraries for the primer pair *g-h*, we first used a combined library of
139 815 arctic species (Sønstebø et al. 2010) and additional 849 boreal vascular plant taxa at the
140 rank of species, subspecies or variety (Brochmann et al. unpublished). We included in the
141 final dataset all sequences with a ≥ 98% match with this reference library. Of the remaining
142 sequences, we included those with a ≥ 98% match to a sequence in a database constructed
143 by extracting P6-loop sequences from the EMBL Nucleotide Sequence Database by using the
144 software ecoPCR (available at http://www.grenoble.prabi.fr/trac/ecoPCR). For the *c-h*
145 primer pair, we used the same taxonomic reference library of arctic and boreal vascular
146 plant species, supplemented with 455 arctic and boreal bryophyte species (Gussarova et al.
147 unpublished). For the *ITS-Fungi* primer pair, we created a reference database by extracting
148 sequences of the targeted region from the EMBL Nucleotide Sequence Database with
149 ecoPCR. From the two unpublished reference libraries, the sequences by which the taxa
150 were identified in this study (n=83 for vascular plants and n=48 for bryophytes) were
151 submitted to the EMBL Database (accession numbers embl:HE993553-ebml:HE993683). For
152 both *g-h* and *c-h* primers the retrieved groups were afterwards compared both with the
153 known regional flora and the reference libraries coverage of all relevant taxa. Details of
154 these taxonomic adjustments are described in Appendix 1. Nomenclature for vascular plants

155 follows the Annotated Checklist of the Panarctic Flora (PAF) (available at:
156 http://nhm2.uio.no/paf/, accessed 15.6.2012).

157

158 The resulting datasets consisted of a count of sequences per taxon per individual Norwegian
159 lemming. For primer pairs *g-h* and *c-h*, we calculated the proportion of different taxa per
160 individual. Even though DNA metabarcoding data for plants probably reflects small rodent
161 diets well (Soininen et al. 2009), some biases may occur (Soininen et al. 2009; Pompanon et
162 al. 2012) and we therefore also report the number of individuals in which a given taxon was
163 found. Because we are not aware of how well the DNA metabarcoding results for fungi
164 reflect relative abundances of taxa, we calculated only the number of individuals in which
165 different fungal taxa were found. We used the *c-h* dataset to compare the proportions of
166 seed plants, ferns and fern allies (i.e. vascular non-seed plants) and bryophytes (i.e. mosses
167 and liverworts) in diets and to assess the proportions of different bryophyte taxa. We used
168 data from primer pair *g-h* to study the proportions of seed plant taxa. We compared diets
169 between habitats, but did no statistical analysis due to low sample size from wetlands and
170 meadows.

171

172 ## Results

173 Mean proportions of bryophytes, ferns and fern allies and seed plants in Norwegian lemming
174 diets were 0.32 (SE 0.05), 0.02 (SE 0.01) and 0.63 (SE 0.05), respectively. Five individuals, i.e.
175 13 % of the animals included in this study, had not ingested any bryophytes. Two of these
176 individuals came from the heath, two from the meadow and one from the wetland habitat.

Among seed plants, grasses (Poaceae, mean proportion 0.49 (SE 0.06)) emerged as the most important group (Table 1, Figure 1). Among grasses, *Avenella flexuosa* was the dominant species, representing 0.67 of grasses and 0.33 of all seed plants in diets. Other relatively abundant groups were sedges (Cyperaceae, mean proportion 0.15 (SE 0.05)), willows (Salicaceae mean proportion 0.09 (SE 0.04)) and forbs of the family Polygonaceae (mean proportion 0.08 (SE 0.04)), especially *Rumex* spp. In addition, a range of different plant taxa was found in small quantities (Table 1 and Supplementary Table S2).

The bryophytes retrieved were dominated by mosses, liverworts being rare (one liverwort species occurred in one individual). The dominant moss family was Dicranaceae and the most frequentspecies was *Dicranum scoparium*, which alone made up 0.20 of mosses in the diets (Figure 2). In addition, sequences belonging to the Dicranaceae at different taxonomic levels (species, genus and family), were frequent. Several non-Dicranaceae mosses were also present, but their abundance was low (Figure 2, Supplementary Table S2).

Diets of individuals from the different habitats seemed to differ in terms of seed plant composition, although all of these differences have to be interpreted with caution due to small sample sizes (Figure 1). The clearest difference between habitats was the dominance of grasses in the heaths compared with a more varied diet in both wetlands and meadows. No similar difference was found for mosses; the Dicranaceae dominated in all habitats

198 (Figure 2). The proportions of mosses in diets were 0.44 (SE 0.06), 0.20 (SE 0.7) and 0.19 (SE
199 0.10) in heath, meadow and wetland habitats, respectively.

200

201 Most fungi that were successfully identified tothe species level belonged to micromycetes
202 (i.e. groups of fungi which do not produce large fruit bodies) (Supplementary Table S3). Only
203 one of the identified fungi (*Caloplaca flavocitrina*), present in one individual, is known as
204 lichen-forming. Three individuals contained no sequences of fungi.

205

206 Discussion

207 We found that Norwegian lemming diet was dominated by grasses, of which *Avenella*
208 *flexuosa* composed more than half, and mosses, mainly of the genus *Dicranum*. In addition
209 to grasses, Norwegian lemmings had ingested a diverse range of other seed plants, whereas
210 the moss component of their diets was less diverse. Diets varied somewhat between
211 habitats in terms of moss proportion and seed plant composition. A variety of fungi were
212 found in the stomach contents, but hardly any of the identified ones belonged to species
213 that are likely to serve as food.

214

215 Notably, our results show a taxonomical precision and diversity of food items which is clearly
216 higher than observed in previous studies on the diet of the Norwegian lemming (Stoddart
217 1967; Hansson 1969; Tast 1991; Saetnan et al. 2009). However, inference of the quantity of
218 each ingested taxon from the number of DNA sequences retrieved should be done with

some caution. The DNA metabarcoding method has been directly compared with the traditional microhistological approach for voles, indicating that the two methods identify similar proportions of food items (Soininen et al. 2009). However, factors biasing the food item proportions may occur in each of the different steps from ingestion by the animal to identification and counting of sequence reads obtained. These factors include differential digestibility of the ingested food species, differences in the barcode copy number per species and bias introduced in the PCR and in the emulsion PCR prior to sequencing, where shorter reads may preferentially be amplified (Engelbrektson et al. 2010) (for a thorough description of DNA metabarcoding methodology for diet analysis and potential errors related to it, see Pompanon et al. (2012)). A conclusive test of how well the ingested food item proportions correspond to the proportions that are detected by the DNA metabarcoding method would necessitate an analysis of a diet of known proportions, but this is outside the scope of the current study.

The general pattern that Norwegian lemmings feed mainly on grasses and mosses during summer has also been found in most other studies (Stoddart 1967; Hansson 1969; Tast 1991). Nevertheless, our results suggest that lemming diet is both more diverse and includes more vascular plant species than previously believed. For example, Tast (1991) states that "Norwegian lemmings feed mostly on mosses in all habitats and seasons when they are available", which is clearly contradictory to our results. Our results suggest that the dominance of grasses and mosses is most pronounced in the heath habitat, and that the diet is more diverse in the meadow and wetland habitats. Such differences in lemming diets between habitats are likely to be attributed to the availability and quality of different food

items (Batzli 1993). However, a larger sample size would be required for investigating whether the observed patterns are consistent, and detailed data on vegetation would be needed for understanding their causes.

Comparison of our results with previous studies suggests that there is regional variation in the feeding habits of the Norwegian lemming. For example, Saetnan et al. (2009) report Norwegian lemming diets dominated by Cyperaceae in "alpine willow thicket-meadow" habitat in central Norway, which resemble the meadow habitats in the current study. We found a quite large proportion of sedges in the diets of Norwegian lemmings caught in meadows as well as in the two other habitats, but grasses and mosses to be generally more important. Further, we found that *Avenella flexuosa* alone formed one third of the seed plants in the Norwegian lemming diets. Previous studies have found variable amount of this grass in Norwegian lemming diets, from being a frequently eaten grass (Hansson 1969) to not being present at all (Saetnan et al. 2009). *Avenella flexuosa* is a common grass in the study area of the latter study, as in our study area (Saetnan et al. 2009; Ravolainen et al. 2013). Thus, difference in availability alone is unlikely to explain the recorded difference in the use of this species. While some of this discrepancy may be explained by low resolution of the microhistological methods, it seems unlikely that this would be the case for such distinct groups as sedges, grasses and mosses. We therefore suggest that in addition to differences in diet between habitats, as suggested by our results, there may be regional differences in Norwegian lemming diet. Such variation may cause lemming-vegetation interactions to differ between habitats and regions and thus cause such an attribute as population outbreak amplitude to exhibit spatial variation (Ims et al. 2011).

The majority of mosses we found in Norwegian lemming diets belonged to the genus *Dicranum*, which is in line with previous findings from both Norwegian lemmings (Kalela et al. 1961; Stoddart 1967; Tast 1991) and wood lemmings (*Myopus schisticolor*) (Eskelinen 2002). Interestingly, Eskelinen (2002) suggested that the high nitrogen content he observed in *Dicranum* could explain such a preference in wood lemmings. On the other hand, Hansson (1969) suggested *Hylocomium splendens* to be the most commonly eaten moss by Norwegian lemmings in northern Sweden. *Dicranum* spp. are generally more frequent in arctic and alpine vegetation than *H. splendens* (Austrheim et al. 2005; Hassel et al. 2012), and high availability may explain the dominance of *Dicranum* spp. in the Norwegian lemming diet. We suggest that either methodology or different abundance or quality of available mosses in vegetation could have caused this discrepancy. This interpretation of between-habitat and -site variability is supported by the findings by Kalela et al. (1961), whose feeding experiments indicate that Norwegian lemmings do not exclusively prefer *Dicranum* spp..

Most macromycetes (i.e. fungi which produce large fruit bodies) in the study area that could serve as food for Norwegian lemmings belong to Agaricomycetes (Hansen and Knudsen 1992), which occurred sparsely in our samples. Instead, the majority of the identified species were micromycetes, plant pathogens, root-associated or saprotrophic fungi. Such fungi are probably eaten passively, with plants (Jensen et al. 2011), or they may be part of the flora in the digestive system of Norwegian lemmings. Whether Agaricomycetes were actually present but undetected, were identified at higher taxonomic levels (most individuals had un-identified fungi in their diet) or were absent because the Norwegian lemmings do not feed

288 on fungi cannot be firmly concluded. As the presence of fungi and plants was analyzed
289 separately, their abundances cannot be compared. Most of the analyzed individuals were
290 collected during autumn, when large fruit bodies of Agaricomycetes are in general most
291 abundant. Even though the macromycetes are more available in the autumn they were not
292 found in Norwegian lemming diets from the same period. We therefore find it unlikely that
293 they would constitute an important part of Norwegian lemming diet during other seasons.
294 Hence, our results support the conclusion of Koshkina (1961), that fungi are unimportant as
295 food for Norwegian lemmings.

296

297 Rather than serving as food, ingested micromycetes are more likely to have implications for
298 food quality of Norwegian lemmings. Many endophytic fungi produce toxins that are harmful
299 for mammals, although certain fungal associates of plants may have also positive effects for
300 small rodents (Saikkonen et al. 1998; Saari et al. 2010). A diverse fungal community is
301 associated with both mosses and grasses, even if the ecology of such interactions is poorly
302 known (Davey and Currah 2006; Kauserud et al. 2008; Jensen et al. 2011). It is thus possible
303 that at least some of the fungi which we found may change the quality of plants as food for
304 Norwegian lemmings. More knowledge of the fungi in Norwegian lemming diets as well as in
305 their food plants is clearly needed to understand their ecological role for Norwegian
306 lemmings. The variable diets of Norwegian lemmings between habitats and regions,
307 suggested by our results, in combination with the variable use of habitats throughout the
308 phases of population cycles (Kalela et al. 1961; Tast 1991), may have implications for the
309 quality of ingested food and thus for the condition of the individual Norwegian lemmings.

310

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Conflict of Interest

We would like to mention that 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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455 **Table 1** Composition of seed plants (mean proportion of DNA sequences of spermatophytes
456 in stomach contents analyzed using *g-h* primer pair) in diets of Norwegian lemmings (n=40)
457 during a population density peak in northern Norway. At each taxonomic level, the
458 contributions from lower levels are presented when known. Column "Frequency" refers to
459 number of lemming individuals from which the taxa was recorded. Column "Change" shows
460 taxa for which the identity was adjusted; "+" indicates that at least part of the sequences
461 included in the taxon were re-assigned to a more specific taxonomic level,"-" the opposite;
462 "F" indicates that this change was done based on the known regional flora and "B" that it
463 was done due to lack of relevant reference species in the databases used. Included are taxa
464 with a mean % > 0.1.

Family	Genus	Species	Mean % (SE)	Frequency	Change
Poaceae			48.8 (6)	40	- F
	Avenella	*Avenella flexuosa*	33.6 (5.1)	37	
	Festuca		3.2 (1.4)	31	
	Poa		0.9 (0.2)	30	
	Anthoxanthum	*Anthoxanthum nipponicum*	0.1 (0)	9	
Cyperaceae			15 (4.5)	26	
	Carex		10.1 (3.3)	23	- B
	Eriophorum		5 (2.4)	14	
Salicaceae			9.2 (4.1)	29	
	Populus	*Populus tremula*	2.4 (2.4)	3	+F
Polygonaceae			7.9 (3.7)	30	
	Rumex		7.2 (3.7)	27	-F
	Bistorta	*Bistorta vivipara*	0.7 (0.3)	26	
Ericaceae			6.1 (2.6)	33	
	Vaccinium		2.5 (1.2)	27	
	Vaccinium	*Vaccinium myrtillus*	1.9 (1)	25	
	Vaccinium	*Vaccinium uliginosum*	0.1 (0.1)	13	
	Empetrum	*Empetrum nigrum* s.lat.	2 (0.9)	18	+F
	Kalmia	*Kalmia procumbens*	1.5 (1.5)	2	
Betulaceae	*Betula*		6.6 (2.4)	28	
Cornaceae	*Chamaepericylum*	*Chamaepericylum suecicum*	1 (0.7)	16	
Caryophyllaceae			0.9 (0.9)	3	
	Cerastium		0.9 (0.9)	2	
		Cerastium fontanum coll.	0.9 (0.9)	1	
Asteraceae			0.7 (0.3)	22	- F
Ranunculaceae			1.1 (0.6)	21	
	Ranunculus		1 (0.6)	20	- F

Juncaceae			1.3 (1.1)	11	
	Juncus		1.3 (1.1)	10	
	Juncus	*Juncus trifidus*	0.7 (0.6)	3	
Orchidaceae	*Listera*	*Listera cordata*	0.3 (0.3)	1	
Rosaceae			0.2 (0.2)	8	- F
	Filipendula	*Filipendula ulmaria*	0.2 (0.2)	4	
Orobanchaceae			0.1 (0)	6	
Violaceae	*Viola*		0.1 (0)	7	
		Viola biflora	0.1 (0)	6	
Classified above family level			1.3 (0.7)		

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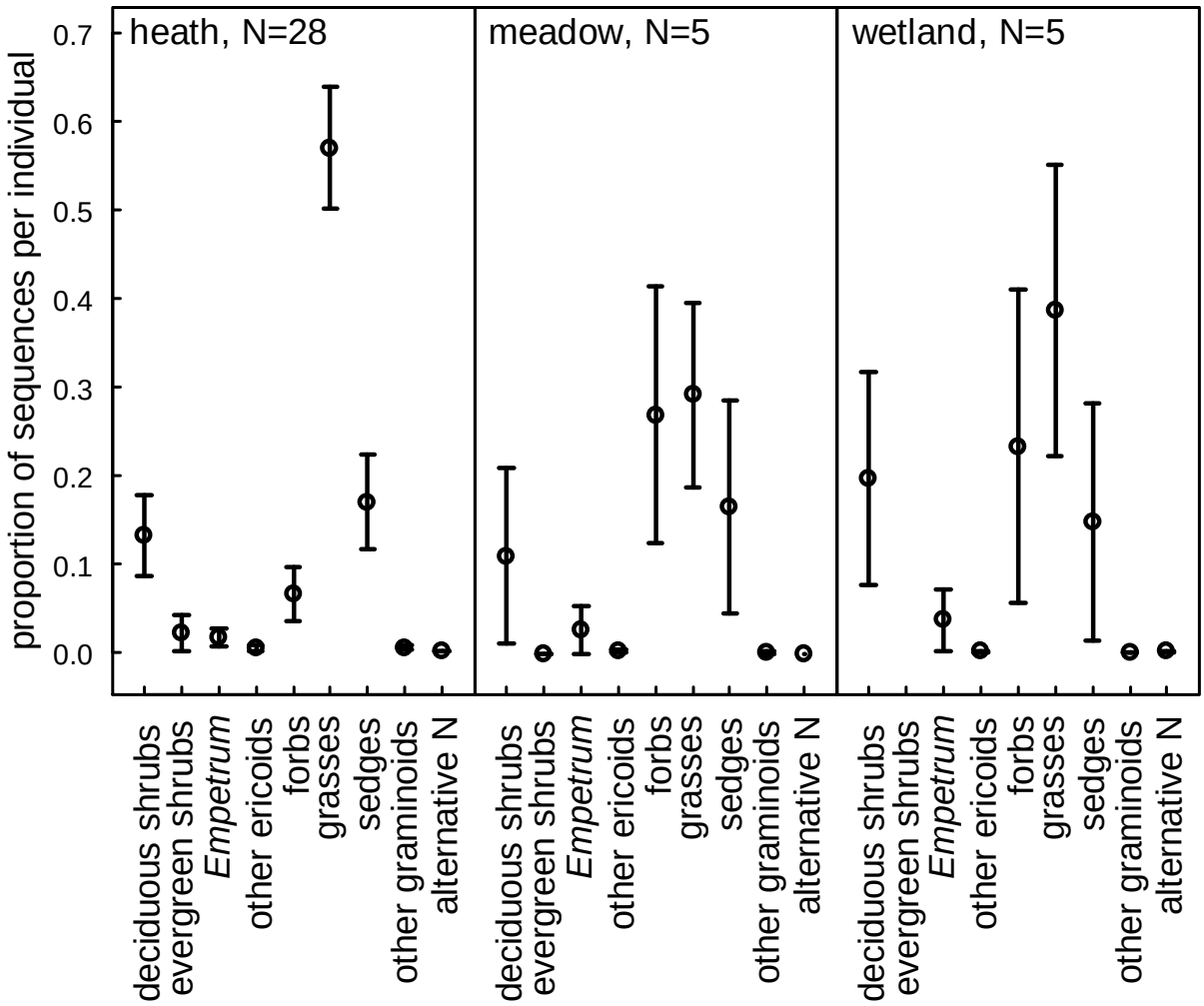
Fig. 1 Proportion (mean and SE) of seed plant sequences per lemming stomach in three different habitats, (using *g-h* primer pair). Category "other ericoids" includes sequences assigned to taxa that contain both deciduous and evergreen ericoid shrubs; category "other graminoids" includes sequences assigned to a taxonomic level which contains both grasses and sedges; category “alternative N” includes hemiparasites and nitrogen fixers.

Fig. 2 Proportion (mean and SE) of moss sequences in lemming diets (using *c-h* primer pair) in three different habitats. At each taxonomic level, the contributions from lower levels are presented when known (e.g. *Dicranum* includes both *D. flexicaule* and *D. scoparum*, as well as sequences assigned to *Dicranum* as a genus). Taxa with only one representative in Fennoscandia are plotted at upper taxonomic level (genus Aulacomnium within family Aulacomniaceae and *Pleurozium schreberi* within genus *Pleurozium*) (Hill et al. 2006).

478 **Fig. 1**



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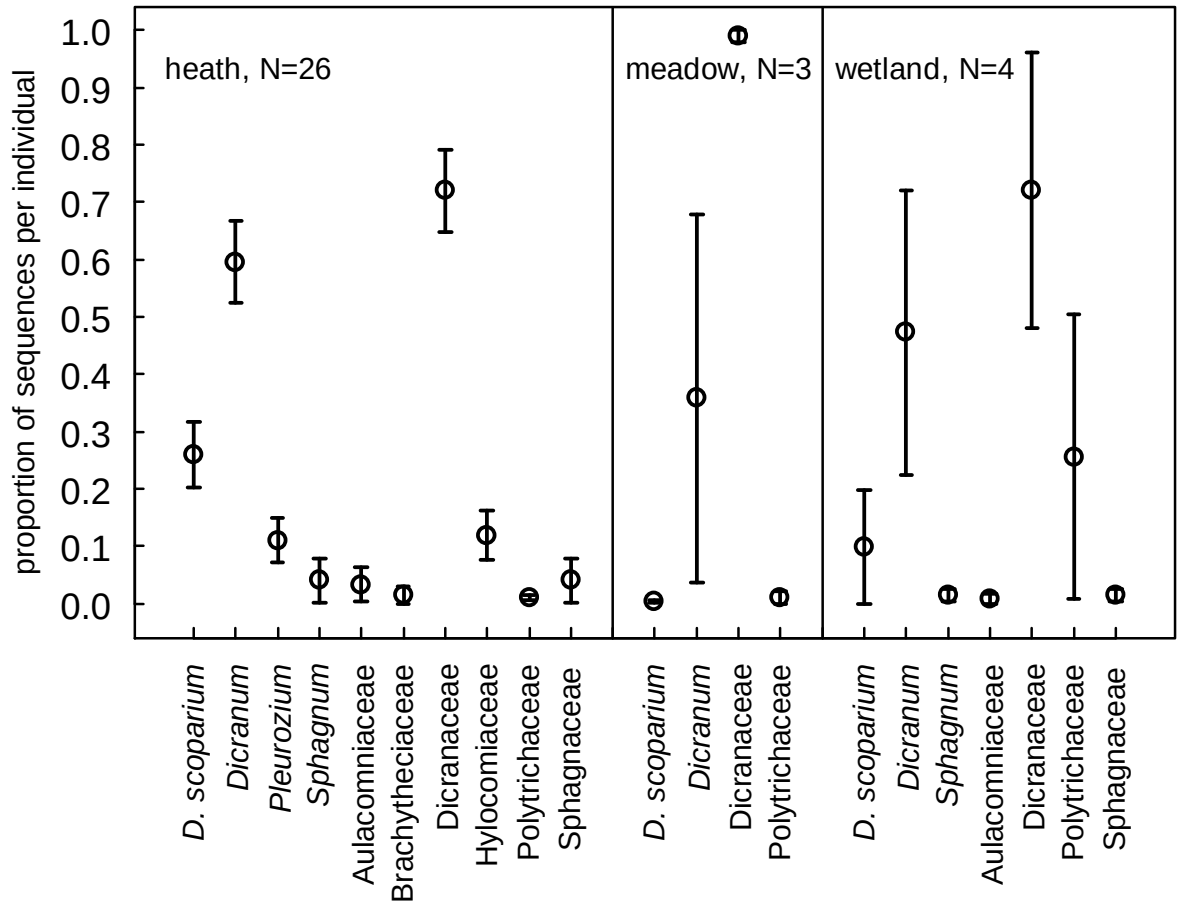
490 **Fig. 2**



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Appendix 1.

Details of taxonomic adjustments

For seed plants, we first verified the taxonomic annotation of sequences based on the region's flora (Lid and Lid 2005, Mossberg and Stenberg 2005, Norwegian Biodiversity Information Centre and GBIF Norway 2012). Several vascular plant genera are represented only by one species in the study area. We therefore attributed sequences assigned to these genera to the respective species (e.g. *Empetrum nigrum, Geranium sylvaticum*). When a species was identified that is not present in the study area and several possible species could come in question, the adjustment was done to a less specific level (e.g. *Euphrasia tatarica* was assigned to genus *Euphrasia*). For each identified taxon, we also checked whether the taxonomic reference library included all closely related taxa possibly present in the area. If this was the case and when possible, sequences of missing taxa available in EMBL were compared to the sequences in the taxonomic reference library. If no unambiguous identification of the retrieved sequences was possible, the identification was moved to a less specific taxonomic rank (e.g. from species to genus). Furthermore, we moved sequences assigned to *Vaccinium ovalifolium* to *Vaccinium myrtillus*, because the former is not present in Europe, but the two have almost identical *g-h* region (accession numbers GQ245635-GQ245641 in EMBL). In total, 99.7% and 0.3% of the sequences included in the final seed plant dataset were identified based on the combined arctic and boreal reference library and reference sequences from EMBL, respectively.

We did similar verifications for bryophytes, i.e. comparison to regional flora (Hill et al. 2006, Norwegian Biodiversity Information Centre and GBIF Norway 2012) and reference library coverage. We changed the taxonomic annotation from species to genera for two taxa. First, we moved *Dicranum flexicaule* to genus *Dicranum*, because its close relative *D. fuscenses* was not included in the taxonomic reference library and we could therefore not inarguably differentiate between these two species. Further, we moved *Sphagnum russowii* to genus *Sphagnum*, as sections are probably the lowest level of true recognition within this genus (Shaw 2000; Shaw et al. 2010).

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Supplementary Table S1 Sequence analysis detailed for each of the three primer pairs used in order of execution. The samples were sequenced as a part of a batch of 192 samples comprised partly of samples not presented in this study. First part of the sequence analysis was done for the whole dataset of 192 samples, using software OBITools (available at http://www.grenoble.prabi.fr/trac/OBITools). Thereafter, a new dataset was composed consisting of lemmings only (focal dataset of each step denoted in the first column).

Dataset		*g-h*	*c-h*	*ITS-Fungi*
Whole dataset	Sequences with an error in the primer		2 errors allowed	
	Sequences with an error in the tag sequence		Removed	
	Sequences with fewer reads discarded		<4	
	Unrealistically short sequences removed, threshold length	8	50	50
	Potential PCR errors discarded (using OBIclean[a]), criteria		clustering threshold 10%	
	GenBank database accessed		16th April 2012	
	Software used for sequence annotation		EcoTag (available as part of OBITools)	
	Minimum match with reference sequence	98%	98%	90%
Final dataset of lemmings	Mean no. sequence reads per sample	2405 (range 23-12510)	581 (range 74-1516)	44 (range 0-225)
	Mean no. taxa per sample in final dataset	15.4 (range 6-27)	8.9 (range 3-16)	3 (range 0-9)
	Sequences assigned to species level	45%	57%	12%
	Sequences assigned to genus level	27%	31%	1%
	Sequences assigned to family level	26%	9%	4%

[a]=OBIclean (included in OBITools) identifies progressive changes of one bp, defines clusters which include a maximum threshold proportion of changed sequences, and keeps the most abundant sequence of the cluster

Supplementary Table S2 Rare plant species and genera recorded in the diets of Norwegian lemmings (N=40) during a population density peak in northern Norway using DNA metabarcoding of chloroplast *trn*L intron. Included are taxa which composed on average < 0.1% of seed plants in diets, determined using primer pair *g-h* and taxa which composed on average < 0.1% of mosses in diets, determined using primer pair *c-h.* See methods for details. Column "Frequency" refers to the number of individuals from which the taxa in question was found. Column "Change" shows taxa which identity was changed based on regional flora; "+" indicates that at least part of the sequences included in the taxon were re-assigned to a more specific taxonomic level,"-" the opposite.

Group	Taxa	Frequency	Change
Seed plants	*Andromeda polifolia*	1	
	Arabis alpina	1	
	Bartsia alpina	5	
	Caltha palustris	4	
	Chamerion angustifolium	1	
	Comarum palustre	2	
	Dryas octopetala	1	+
	Geranium sylvaticum	5	+
	Geum rivale	1	+
	Lathyrus pratensis	1	
	Linnaea borealis	1	+
	Lotus corniculatus	1	
	Melampyrum pratense	1	
	Parnassia palustris	1	+
	Phalaroides arundinacea	1	
	Pinus sylvestris	4	+
	Saussurea alpina	5	
	Trientalis europaea	6	+
	Trollius europaeus	2	+
	Vaccinium vitis-idaea	4	
	Alchemilla	3	
	Calamagrostis	7	
	Epilobium	1	
	Euphrasia	1	-
	Galium	2	
	Larix	2	
	Luzula	1	
	Plantago	1	
	Rhinanthus	1	
	Stellaria	1	
	Papaver	2	
Bryophytes	*Hylocomiastrum pyrenaicum*	1	
	Hylocomium splendens	3	

Kiaeria glacialis	1
Lophozia wenzelii	1
Pohlia wahlenbergii	1
Saniona uncinata	1
Bryum	2
Sciuro-hypnum	2

Supplementary Table S3 Fungal taxa ingested by Norwegian lemmings (N=40) during a population density peak in northern Norway, determined with the primer pair ITS5 and 5.8S_fungi on stomach content DNA. Sequences identified to lower taxonomic levels are included at the higher levels. Frequency: number of individuals in whose stomach content DNA-sequences of a taxon. Size class indicates to which fungal size class (micromycete/ macromycete) the taxa belong.

Division	Class	Family	Species	Frequency	Size class
Ascomycota				21	
	Dothideomycetes			4	
		Venturiaceae		3	
			Venturia sp.	2	micro
			Venturia atriseda	1	micro
		No rank		1	
	Leotiomycetes			9	
		Helotiaceae		1	
			Gremminella sp.	1	micro
		Thelebolaceae		8	
	Eurotoimycetes	Herpotrichiellaceae	*Cladophialophora minutissima*	3	micro
	Lecanoromycetes	Teloschistaceae		1	
			Caloplaca sp.	1	micro
			Caloplaca flavocitrina	1	micro
	Saccharomycetes			4	
		Dipodascaceae		4	
			Galactomyces geotrichum	1	micro
			Yarrowia lipolytica	3	micro
Basidiomycota				17	
	Exobasidiomycetes	Exobasidiaceae	*Exobasidium rostrupii*	3	micro
	Agaricomycetes	Schizophyllaceae	*Schizophyllum* sp.	2	macro
	Tremellomycetes	No rank Tremellales		2	
			Trichonosporales sp. LM547	2	micro
	no rank			14	
		No rank Leucosporidiales	*Leucosporidium*	1	micro
		No rank		13	
			No rank	4	
No rank Fungi				38	