Investigating trophic interactions among herbivorous species in a rapidly changing Arctic tundra using DNA metabarcoding

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Abstract

In the Arctic tundra several different species of herbivores co-exist, with potentially large effects on tundra ecosystem functions and dynamics. Understanding the herbivores trophic interactions therefore provides essential knowledge, especially at a time when the Arctic terrestrial ecosystem is under radical changes due to the continued climate warming.

In the first part of my thesis, I wanted to establish how the detectability of DNA changes along the digestive tract of ptarmigans, with my primary objective to test the hypothesis that fecal samples is the most representative sample type and thus can provide the most comprehensive diet analysis. DNA metabarcoding was used to compare four different digestive samples (crop, gizzard, fecal samples from the large intestine, and fecal samples from the ceca). On overall the fecal samples (from the large intestine and the ceca) combined contained the highest percentage of the total detected dietary items (90%), as compared to the samples from crop and gizzard combined (67.5%). However, when excluding fecal samples from the ceca, approximately 13% fewer dietary food items were detected from the fecal samples from the large intestine (79.7%) than from the crop and gizzard samples combined (93.2%). Despite this decrease, fecal samples from the large intestine are still a good representative sample type for dietary analysis in ptarmigans, as most of their diet diversity were captured.

In the second part of my thesis, DNA metabarcoding was used to study diet composition and overlap of five sympatric herbivores in Finnmark, Norway. The herbivores included in this part are ptarmigans (*Lagopus* spp.), reindeer (*Rangifer tarandus*), hares (*Lepus timidus*), moose (*Alces alces*) and rodents. Diet analysis of the herbivores field-collected fecal samples, using the vascular plant primers G/H, detected that the genus *Betula*, shrubs and ferns, were the most common diet categories, followed by mosses, graminoids and forbs. However, the diet composition of individual species varied. For ptarmigan and reindeer, *Betula* was the most dominating diet item. In hare and rodent diet, shrubs were dominating. While ferns were dominating in the moose diet, followed by shrubs. Within shrubs, four dietary items were detected with higher frequency of occurrence. The dwarf shrub genus *Empetrum* was detected in ptarmigan and rodent diet. The dwarf shrub species *Vaccinium ulignosum* and genus *Vaccinium* were detected in ptarmigan, reindeer, hare and rodent diet. Whilst the shrub tribe

Saliceae were detected in ptarmigan, hare and moose diet. Thus, by successfully using field-collected fecal samples, multiple shared MOTUs between the herbivores were detected. These results indicate that high niche overlap exist between the studied herbivore species, but to a lesser extent when including moose.

Table of contents

Acknowledgements III

Abstract V

General introduction 1

1 Comparing digestive samples from ptarmigan using DNA metababarcoding 3

1.1 Introduction 3

1.2 Material and methods 6

1.2.1 Study sites 6

1.2.2 Sample collection and handling 7

1.2.3 Molecular diet analysis 9

1.2.4 Bioinformatic analyses 11

1.2.5 Data filtering and statistical analyses 13

1.3 Results 15

1.3.1 Comparison of results obtained with the four primer sets 15

1.3.2 Results obtained with the G/H primers 15

1.4 Discussion 20

1.5 Conclusion 22

2 Trophic interactions between herbivore species in Finnmark 23

2.1 Introduction 23

2.2 Material and methods 25

2.2.1 Study sites 25

2.2.2 Sample collection and handling 27

2.2.3 Molecular diet analysis 28

2.2.4 Bioinformatic analyses 29

2.2.5 Data filtering and statistical analyses 29

2.3 Results 33

2.3.1 Comparison of results obtained with the four primer sets 33

2.3.2 Results obtained with the G/H primers 34

2.4 Discussion 39

2.5 Conclusion 42

General conclusion 43

References 45

Appendix A – Comparing digestive samples from ptarmigan using DNA metabarcoding ... 51

Appendix B – Trophic interactions between herbivore species in Finnmark 61

General introduction

Atmospheric temperatures have risen dramatically over the last decades (Huang et al., 2017), with higher rises in surface air temperature in the Arctic compared to the Northern Hemisphere's average (Serreze et al., 2009; Bintanja and Linden, 2013). The continued warming is expected to radically transform Arctic terrestrial ecosystems (Henden et al., 2016), and is already beginning to negatively impact Arctic terrestrial food webs, especially endemic Arctic species (Meltofte, 2013).

The Arctic tundra is home of numereous herbivores, such as ptarmigans (*Lagopus* spp.), reindeer (*Rangifer tarandus*), hares (*Lepus timidus*), moose (*Alces alces*) and various species of rodents. All of these herbivores are sympatric and belong to the same trophic guild, meaning that they share both food plants and habitats, which might also trigger competition among the species (Henden et al., 2017). Ptarmigan, for instance, is among the most intensively studied birds worldwide (Moss et al., 2010; Henden et al., 2017), with its populations being under constant monitoring. Observations show that the Norwegian ptarmigan populations have decreased over the last decades (Shimming and Øien, 2015), whilst reindeer and moose (*Alces alces*) populations in Finnmark have showed the opposite trend (Henden et al., 2011). However, determining which are the main factors driving these dynamics is not very clear yet. The combination of climate warming with changes in food plant quality and distribution could play an important role (Fauchald et al., 2017: Leclerc et al., 2019).

By investigating the Arctic herbivores diet and niche overlap among species, we can get valuable information in terms of their respective dietary preferences and the potential for competition among different species, as well as their respective impact on different types of vegetation. This is important knowledge that could help anticipate climate change effects on both Arctic herbivores and plant communities.

It has already been documented that vascular plant biomass have increased rapidly, and in some areas the timing of flowering has advanced up to almost one month compared to a decade ago (Meltofte, 2013). Several studies have also shown that ungulate browsing in the tundra is capable of reducing both willow cover and height (Henden et al., 2011, and

references within). This may pose a risk for ptarmigans, as they are known to rely on willow shrubs (Saliceae) for both food and cover (Henden et al., 2011, and references within).

Studying diet of multiple species of herbivores could be challenging, but the recently developed method DNA metabarcoding has proved to be efficient in characterizing highly diverse diets in a range of species (Kartzinel et al., 2015), including tundra herbivores such as rodents (Soininen et al., 2014). DNA metabarcoding usually relies on the use of general primers, high-throughput sequencing and environmental DNA samples such as animal feces collected from the field (Valentini et al., 2009). However, we still do not know whether feces are the most representative sample type for inferring herbivore diet.

In my first chapter, I took advantage of an existing collection of ptarmigan samples, in order to validate whether feces were a representative sample type for inferring herbivores diet. In order to study diet analysis within herbivores, knowing which type of sample is the most representative of the herbivore diet is crucial in order to get the most comprehensive diet analysis with the use of DNA metabarcoding. In my second chapter, I used field-collected fecal samples to study niche overlap between ptarmigan, reindeer, hare, rodent and moose, all of which are found in the Arctic tundra in Finnmark, Norway.

1 Comparing digestive samples from ptarmigan using DNA metababarcoding

1.1 Introduction

Studying diet and trophic interactions in wild animals can be extremely challenging for ecologists. The main reason is the limited ability of determining accurately, but also efficiently in terms of time and costs, the complex variety of food types that animals can feed upon (De Barba et al., 2014). Consequently, new and more accurate techniques are needed for establishing the precise identity of consumed taxa, but also the relative proportions in which they are ingested (Pompanon et al., 2012).

In recent years, DNA metabarcoding has become a popular tool for studying animal diet (Gebremedhin et al., 2016; Hawlitschek et al., 2018). With this approach we only need minimal priori knowledge of the possibly consumed foods, in order to be able to select for the most appropriate metabarcoding primers. Another main advantage of DNA metabarcoding is that it allows to simultaneously analyse several thousands of samples in a single sequencing run (Valentini et al., 2009; Coissac, 2012; De Barba et al., 2014) making it an extremely cost-effective method for diet analysis. Based on these advantages, DNA metabarcoding has proven to be a valuable technique for analysing the diet in a large number of small and large herbivore species, detecting multiple dietary items, even in species where current knowledge of their diet is limited (Soininen et al., 2013; Kartzinel et al., 2015; Gebremedhin et al., 2016). For all these species, results show that compared to other methods for diet analysis, such as direct visual observations or microhistology, DNA metabarcoding is more precise in terms of diet estimation (Nichols et al., 2016). In addition, direct observation and microhistology can be excessively time-consuming in order to be viable techniques (Collopy 1983). This is especially relevant in the case of birds, which are extremely mobile and it may therefore be difficult to accurately identify their diet through direct observations (Red, 1994). Microhistology has also limitations as it greatly depends upon the personal skills and training, as well as the extend of degradation of food items, for visually identifying prey species from digested food remains from gut contents or feces (Pompanon et al., 2012). However, DNA metabarcoding might show some disadvantages, as well. For instance, Nichols et al. (2016) showed that taxa present in feces from ungulates, which had been identified with

microhistology, were sometimes not detected with DNA metabarcoding. In addition, DNA metabarcoding requires access to a comprehensive database for matching prey sequences in order to get precise taxonomic identifications.

To my knowledge, only a few studies exist that have used DNA metabarcoding for bird diet analysis (e.g. wood grouse, Valentini et al., 2009; penguins, Deagle et al., 2010). Ptarmigans are an important part of the arctic food web as they are found continuously distributed in the low-arctic tundra, the sub-arctic tundra and the high-arctic tundra (Henden et al., 2017), yet there are none DNA metabarcoding dietary studies focusing on ptarmigans while many other arctic herbivores have already been studied (e.g. barnacle goose, Stech et al., 2010; lemmings, Soininen et al., 2013; Soininen et al., 2015; small rodents, Soininen et al., 2014).

Herbivorous birds can have a quite complex digestive system. After a food item has been swallowed, it goes directly into a very thin and expandable sack, called the crop. In the crop, the food can be stored for some time before it starts being digested (Stokkan, 1992). In the gizzard, the food gets grit down by very small stones, which the ptarmigans regularly intake throughout the year (Norris et al., 1975). After the digestion process, the remainings go either straight out through the large intestine or into the ceca. In the ceca, fluid has time to be absorbed and further digestion of food may take place, but the functioning of ceca is only partly understood (Clench and Mathias, 1995). How this complex digestive system influences DNA metabarcoding diet analysis in herbivorous birds in general, and in ptarmigans specifically, it is yet unknown. Therefore, for this chapter I have sampled four different digestive samples (crop, gizzard, feces from the large intestine and feces from the ceca) obtained from ptarmigans.

Feces are usually an easily obtainable source, which can be sampled without interfering with the animal itself when the feces are field-collected. Therefore the sampling of feces allows for sampling food webs without disturbing the system, as it is a non-lethal technique. It is then an excellent sampling method for anyone working with conservation and management, and will be a good approach especially for species in decline. If it is shown that feces can provide a representative diet estimate of ptarmigans diet, then DNA-based analysis from feces could be potentially used for any other bird species with similarly complex digestive system. On the other hand, the quality of the DNA obtained from fecal samples collected from the field are often much lower than other samples from the digestive tract due do both digestion and decomposition (Hawlitschek et al., 2018, and references within). Therefore, the crop and the

gizzard digestive samples may give better and more correct information about the ptarmigan diet, especially samples from the crops where the foods only are swallowed before any digestion or grinding has yet started. With the use of DNA metabarcoding, I will in this chapter answer the following question: A) Are feces, especially feces from the large intestine, representative samples of the ptarmigan diet?

1.2 Material and methods

1.2.1 Study sites

Three ptarmigan species were studied, 15 individuals of the willow ptarmigan (*Lagopus lagpus*) and 8 individuals of the rock ptarmigan (*Lagopus muta*) in Norway and 8 individuals of the Svalbard rock ptarmigan (*Lagopus muta hyperborea*) in Svalbard (see appendix A1, Table A1.1). 18 out of the 23 ptarmigan individuals of the two Norwegian species were shot between October and November 2015 in the surroundings of Tromsø, Norway. They were most likely shot in the Kvaløya or Ringvassøya areas (Fig. 1.1), but the exact locations were kept secret by the hunters that shot them. Two individuals (lm_c and lm_004, see appendix A1, Table A1.1) were shot on 29 October 2016 in the surroundings of Tromsø, one individual (ll_022, see appendix A1, Table A1.1) were shot on 29 November 2016 in the surroundings of Tromsø, while two other individuals (lm_001 and ll_016, see appendix A1, Table A1.1) were shot at unknown dates (but most likely between October 15th and December 23rd, 2014) at Kvaløya. All of the Svalbard rock ptarmigans were shot at Akseløya in October 2015 (Fig. 1.2).

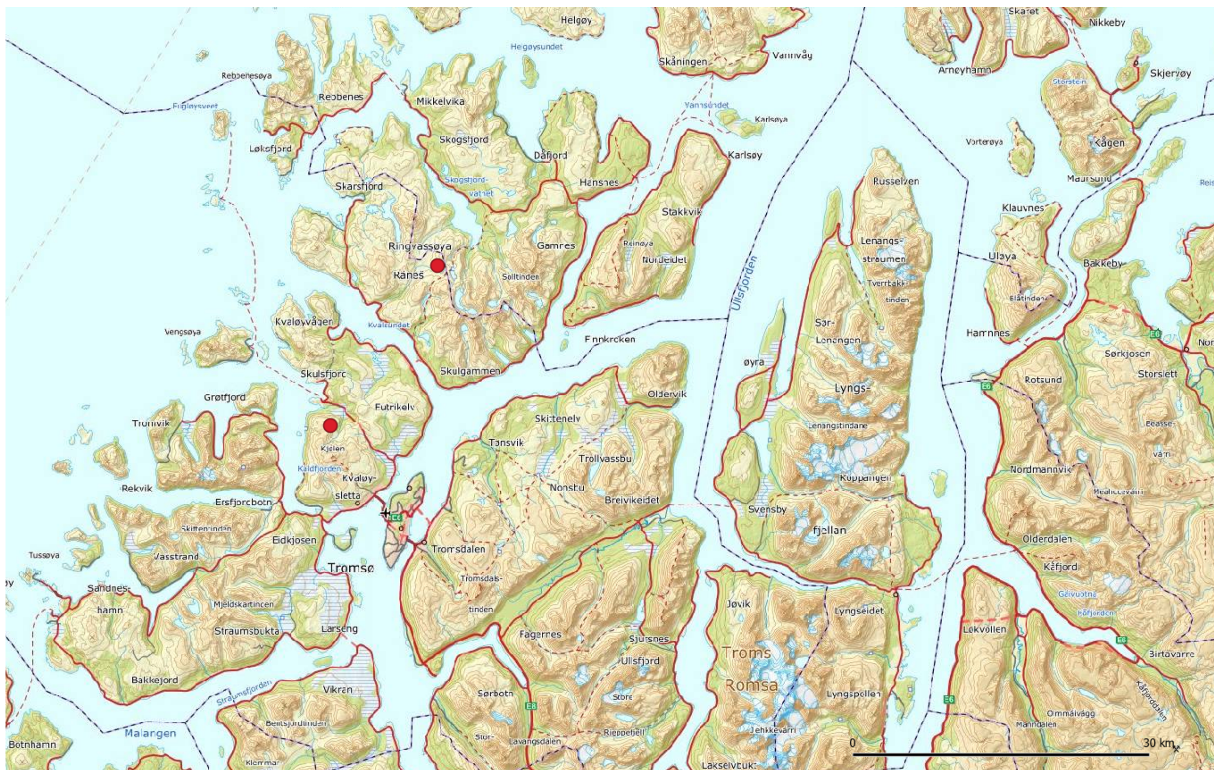


Figure 1.1. The two Norwegian species, willow ptarmigan and rock ptarmigan, were shot in the surroundings of Tromsø, with the two islands Kvaløya and Ringvassøya as the two most likely hunting locations. Red dots do not

represent the exact location. Map was made in QGIS v.3.4 (https://qgis.org), by downloading the map package "Matrikkel Bagrunn2" from Geonorge (https://www.geonorge.no).

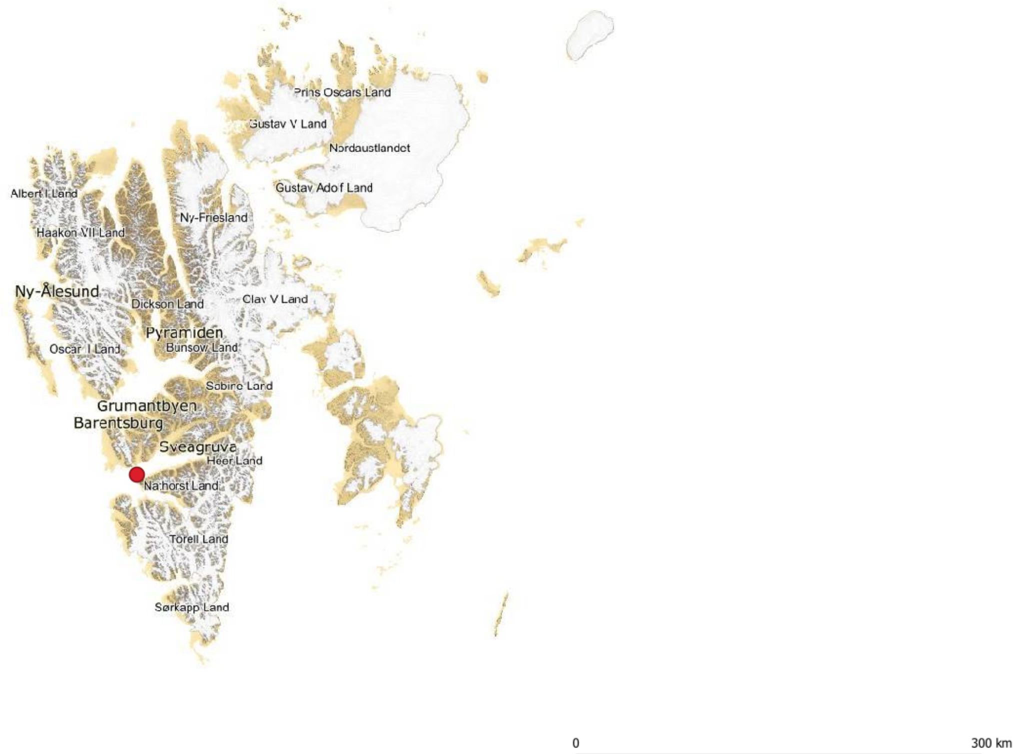


Figure 1.2. All Svalbard rock ptarmigan individuals have been shot and collected at Akseløya, Svalbard, in 2015. Map was made in QGIS v.3.4 (https://qgis.org), by downloading the map package "Matrikkel Bagrunn2" from Geonorge (https://www.geonorge.no).

1.2.2 Sample collection and handling

After the hunters had shot the *Lagopus* specimens, the birds were most likely hung in outside conditions for a couple of days in Tromsø, and up to a week in Svalbard, before being stored at -20°C. The specimens were later delivered intact to Andreas Nord (University of Tromsø), who stored the specimens at -20°C to -25°C. Prior to dissections, the birds were thawn overnight at 15°C. The entire digestive system of every bird was removed, packed in dry ice and shipped to Jennifer Forbey´s lab at Boise State University, USA. During dissections, gloves were changed between every second bird, and dissection blades and scissors were wiped down between every bird. For each bird, the digestive system was cut at the esophagus,

and the connective tissue between gut sections was also cut when needed. However, the integrity of the digestive system was untouched.

At Jennifer Forbey´s lab, the specimens were thawed one more time and the different digestive system contents dissected by also cutting of crop and/or gizzard for a few specimens (see appendix A2, Table A2.1). In these cases, the equipment was not sterilized between the dissections of the different digestive parts. In addition, some of the ptarmigan specimens were further manipulated by an undergraduate student (see appendix A2, Table A2.2). The student unwounded the whole digestive track for each manipulated specimen in order to get its total length measured. During the process, the crop and gizzard´s integrity was preserved. Only the large intestine may have been potentially contaminated at its end. But the student changed gloves between every individual, thus limiting the probability of contamination occurring among bird specimens.

After this, and until I arrived at Jennifer´s lab, the ptarmigan digestive tracts were stored continuously at -20°C. In January 2018, I spent almost two weeks at the lab, in order to retrieve the ptarmigan digestive tract contents. I dissected both the crop and the gizzard in order to subsample their digested food remains. For this, I separated the crop and the gizzard from the remaining gut system, using a clean scissor. Then, for each gizzard, I used a clean scalpel with a disposable blade to cut the gizzard in almost two halves. After this, I pulled the gizzard muscles backwards and squeezed the contents into a new clean plastic weight boat at my dissection sheet. Using a clean spoon, I scraped out the rest of the contents from the gizzard. From the plastic weight boat, I used the same spoon to transfer everything into a small labeled zip-lock bag. The concent was then weighted by putting the zip-lock bag into the weigh boat placed on the scale. Then, with the same spoon, I took out approximately half of the content into a clean paper teabag, which was put into a larger labeled zip-lock bag prefilled with beads of silica gel (Carl Roth, Germany) that I brought back to the University of Oslo. The other half of the contents was stored at -20°C at Jennifer Forbey´s lab for further analyses (e.g. microhistology). For the crop samples, I located the connection where I previously cut the crop from the rest of the gut tract. Then with my thumbs covered with gloves, I could easily unfold the crop and let the contents drop into a clean weight boat. I also scraped out the remaining contents using a clean spoon. The rest of the procedure was the same as described above.

Between each sample, the tared weight boat inside the scale was always changed with a new clean one. In addition, all instruments were soaked in 10% bleach solution and then sterilized for a few minutes using a Bunsen burner between each sample. I also took specific care to minimize external contamination by using protective goggles and a facemask, and by changing gloves frequently.

I also subsampled fecal material from the large intestine, and for nine of the willow ptarmigans also from one of the two ceca at the end of the digestive tract. For this, I carefully squeezed out the fecal pellets from the large intestine into a clean plastic scintillation vial prefilled with silica gel beads. The same procedure was applied for the subsampling of the ceca, by first cutting the end of the ceca, and using another clean plastic scintillation vial prefilled with silica gel beads.

Subsamples from crops and gizzards were stored in clean zip-lock bags prefilled with silica gel, while fecal subsamples were stored in sterile 1.5-ml micro-centrifuge tubes prefilled with silica gel. All samples were frozen at -20°C, then kept frozen in dry ice during transportation from USA to Norway, and immediately stored at -19°C at the University of Oslo.

1.2.3 Molecular diet analysis

1.2.3.1 Sample preparation and DNA extractions

All samples were left to completely thaw at room temperature for a few hours. After defrosting, all crop and gizzard contents were homogenized into a fine powder using ceramic mortar and liquid nitrogen. For each sample, 20 mg of the homogenized powder were subsampled for DNA extraction. The required amount was transferred into a new labeled and sterile 2-ml micro-centrifuge tube with a disposable spatula (Chemglass, UK). Sterile, empty micro-centrifuge tubes were held open during sample homogenization in liquid nitrogen in order to monitor for cross-contamination from powder particles. For the fecal samples, I directly withdrew 100 mg per sample and transferred it into a new sterile 2-ml centrifuge tube using disposable spatulas. Subsampled material was stored at -20°C prior to DNA extraction.

Total DNA from all subsamples was extracted using the NucleoSpin Plant II (Machery-Nagel, Germany) according to manufacturer’s instructions. DNA was eluted in a total volume of 100

μl and stored at -20°C. Negative controls (no DNA) were systematically included in order to monitor for potential cross-contamination.

1.2.3.2 PCR amplification and high-throughput sequencing

PCRs were carried out for all samples. Four universal markers were used (Table 1.1), to amplify vascular plants, eukaryotes, fungi and bryophytes, respectively. The markers used were the P6-loop of the chloroplast trnL for detecting vascular plants (G/H; Taberlet et al., 2007) and bryophytes (Bryo01; Epp et al., 2012), 18S rDNA for detecting eukaryotes (Euka02; Guardiola et al., 2015), and ITS1 nuclear rDNA for detecting fungi (Fung01; Epp et al., 2012). These primers have shown high specificity to the target group (Bryo01 and Fung01; Epp et al., 2012), in addition to being highly conserved (G/H; Taberlet et al., 2007) and can assess the biodiversity of all eukaryotic groups (Euka02; Guardiola et al., 2015).

Table 1.1. Overview of the four universal markes used for the DNA amplification. The table includes the targeted taxonomic groups, the primer names, the target genomic regions, the forward primers and the reverse primers.

Taxonomic group	Primer name	Target genomic region	Forward primer	Reverse primer
Eukaryotes	Euka02	18S rDNA (V7)	TTTGTCTGSTTAATTSCG	TCACAGACCTGTTATTGC
Vascular plants	G/H	P6 loop trnl	GGGCAATCCTGAGCCAA	CCATTGAGTCTCTGCACCTATC
Bryophytes	Bryo01	P6 loop trnl	GATTCAGGGAAACTTAGGTTG	CCATYGAGTCTCTGCACC
Fungi	Fung01	ITS1 nuclear rDNA	GGAAGTAAAAGTCGTAACAAGG	CCAAGAGATCCGTTGYTGAAAGT

Each PCR reaction consisted of 2μl DNA, 4.15 μl H_2O, 7.5 μl AmpliTaq Gold Master Mix, 0.6 μl BSA and 0.75 μl Primer mix, giving a total of 15 μl. The concentrations for the PCR reagents were the following; 1x mM AmpliTaq Gold Master Mix, 0.4 mg/ml BSA (10 mg/ml) and 0.5 μM Primer mix F & R (10 μM). The cycling conditions for vascular plants, eukaryotes, fungi and bryophytes are listed in appendix A3 (Table A3.1-A3.4).

On every 96-well plate, at least two PCR negative controls were included (i.e. containing water instead of DNA). These negative controls were used to identify potential contaminations during the PCR procedure. In addition, on each of the 96-well plates amplified with the vascular plant (i.e. G/H) primers, two positive controls were included. The positive controls consisted of a mixture of artificial plant DNA sequences with known

concentrations. These artificial sequences were designed by Peter Heintzman (2019, in prep.) based on GC-content and amplicon lengths of real trnL P6-loop plant sequences.

Every PCR reaction containing the G/H primers and the Euka02 primers were replicated three times, whilst every PCR reaction containing Bryo01 primers and Fung01 primers was run only once. The replication was done in order to ensure that the results were reproducible. In order to prepare the samples for high-throughput sequencing, all the PCR products were pooled together in two tubes. One of the pools contained all the PCR products amplified with the vascular plant and bryophyte primers, and the second pool contained all the PCR products amplified with the eukaryote and fungi primers. However, to guarantee that we could assign sequences to each sample after pooling and high-throughput sequencing, we used primers that were individually tagged with identical forward and reverse 8-9 nucleotides sequence tags. The pooled samples were then purified using the QIAquick PCR Purification Kit (Qiagen, Germany), and checked on a 1.5% gel electrophoresis. Purified DNA was quantified using Qubit® 2.0 fluorometer with the dsDNA HS Assay kit (Invitrogen, Life Technologies, Carlsbad, CA, USA), prior library preparations and sequencing. The purification and quantification were done by S. Kamenova (postdoc, REININ project). The final pools were sent to the Norwegian Sequencing Center for 150bp pair-end sequencing using a Illumina HiSeq 4000 machine (Illumina, USA).

1.2.4 Bioinformatic analyses

Number of raw sequences produced per library is given in Table 1.2. The bioinformatic analyses were analysed using the OBITools software (http://metabarcoding.org/obitools) on Abel (https://www.uio.no/english/services/it/research/hpc/abel), a high performance computing cluster, which uses a Linux operating system. First step consisted in aligning and merging together forward and reverse pair-end reads using *illuminapairend* programme in OBITools. Reference files were made for each library. Each line in the reference files contained information about the primer type (i.e. G/H, Bryo01, Euka02 or Fung01), the name of the sample and its replicate number (i.e. replicate 1, replicate 2 or replicate 3), the primer tag, and the forward and the reverse primer. The reference files were structured in the same way as the 96-well plate, meaning that the first line represent A1 on the PCR plate and the second line represent A2 on the PCR plate (i.e. the PCR plate were followed vertically, starting on the top left and ending on the down right). Then, the reference files together with

the input file containing sequences were analysed with the *ngsfilter* command, where primer and tag combinations were identified and matched to their corresponding sample. Only sequences with a 100% match on tags and with a maximum of two errors in primers were retained for further analyses.

Table 1.2. Overview of the libraries and their content after the receiving of the raw sequences from the Norwegian Sequencing Center.

Library ProcessID	Library content	Number of raw sequences
Lib4	GH+BRYO PtarmUS	19 072 455
Lib8	EUKA+FUNGI PtarmUS	19 221 469

The following step involved discarding all the potential erroneous sequences using the *obigrep* command. The sequence tags that did not have a 100% match were discarded. Thereafter, *obiuniq* was used for merging together all strictly identical sequences, while still keeping the information about their distribution among the samples. The command *obigrep* was then used again in order to remove all the sequences occurring only once (singletons) and sequences with a count lower than five reads, and/or shorter than 10 base pairs (bp). These numbers roughly correspond to the minimum number of artifactual sequences to be removed from the dataset, without a priori (De Barba et al. 2014). Finally, with the use of *obiclean* all potential PCR errors were flagged.

Sequences were taxonomically assigned using the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database (https://www.ncbi.nlm.nih.gov) for all the primer sets using *ecotag*. In addition, a custom reference library (called Ecochange) containing 815 arctic and 835 north boreal vascular plant species (Sønstebø et al., 2010, Willerslev et al., 2014), and 455 bryophyte species (Soininen et al., 2015) were used for improving taxonomic assignation with the G/H primers. Then, all final tabulated files for each primer set were used for data filtering and statistical analyses.

1.2.5 Data filtering and statistical analyses

The data filtering and statistical analyses were done in R, R software v.3.3.2 (R Core Team, 2016), using Rstudio. Results for each of the primers was filtered and analysed separately.

G/H dataset

G/H tabulated files based on taxonomic assignations from EMBL and Ecochange reference databases were imported and analysed together using ROBITools package (https://git.metabarcoding.org/obitools/ROBITools). First step of filtering consisted of the removal of all sequencue clusters, called MOTUs (molecular operational taxonomic units (Ryberg 2015)), flagged as PCR errors, which were the MOTUs that appeared with ”internal” status in OBITools (Boyer et al. 2016). Next step was the removal of all sequences with below 65% identity match (i.e. the match of a sequence to the reference database) in order to not eliminate too many real sequences, with the EMBL database, as well as the removal of MOTUs represented with less than 1% of the total number of reads in a PCR (i.e. rare MOTUs). This resulted in the majority of sequences having a 100% match with the EMBL database, a few having 98% match and two having between 94-96% match.

All samples were subdivided into different categories (e.g. ”Birds” and ”Controls”), in order to easily extract the sample information needed during the analyses. With the use of *ggplot* (with the use of tidyverse and gglplot2 packages) I could visualize the number of reads and MOTUs, in each category. The category “Controls”, containing all types of controls, was further investigated. Twelve MOTUs (see appendix A4, Table A4.1) were detected as being more frequent (counts between 47 and 9333) in the negative controls compared to the experimental samples, and were thereby removed entirely from the dataset. Still, three types of negative controls (mortars, liquid nitrogen and an empty tube staying open during the procedure) were showing reads. The positive controls were detected as expected, thus confirming that the PCR reactions were successful.

Next step consisted of looking into the PCR replicates. The first inspection showed mostly none or low distances between the corresponding replicates. All outliers were discarded from the dataset, and only highly similar PCR replicates were retained. The remaining replicates were then merged together. In total, six individuals were included in the final comparison, with all of them belonging to willow ptarmigan.

The frequency of occurence of a MOTUs detection in the different sample types were calculated in R. In addition to using the *setdiff* (set differences of subets) function in R, in order to set differences between percentage of MOTUs found in feces from the large intestine and feces from the ceca.

Euka02 dataset

The same procedure was done for this dataset. The majority of the sequences had a 97-100% best match with the EMBL database. A total of 73 MOTUs (see appendix A4, Table A4.2) were detected as being more frequent (counts between 21 and 9779) in the negative controls compared to the experimental samples, and were therefore removed entirely from the dataset. The procedure was the same as for the G/H dataset for the PCR replicates as well.

Fung01 dataset

The same procedure was done for this dataset. The sequences were almost evenly distributed between 65-100% best match with the EMBL databse, but still more sequences appeared at 100% match. Eleven MOTUs (see appendix A4, Table A4.3) were detected as being more frequent (counts between six and 1119) in the negative controls compared to the experimental samples, and were therefore removed entirely from the dataset. The dataset was not further looked into, as replicates were not made with the Fung01 primers.

Bryo01 dataset

The same procedure was done for this dataset. The majority of the sequences had a 100% match with the EMBL database. None MOTUs were detected as being more frequent in the negative controls compared to the experimental samples, and only one negative control in total were detected in the dataset. The dataset was not further looked into, as replicates were not made with the Bryo01 primers.

1.3 Results

1.3.1 Comparison of results obtained with the four primer sets

The top 20 MOTUs detected with each of the primer sets used are given in appendix A5 (Table A5.1-A5.4). The diet composition retrieved with G/H and Euka02 primers included a range of vascular plant taxa with a 100% identity match to sequences in the reference databases. However, the dataset amplified with the use of Euka02 primers identified also Atlantic herring (*Clupea harengus*) with a 100% identity match, which constituted almost 1% of the total findings amplified with this primer. In the top 20 list using the Fung01 primers, only *Thelebolus*, *Thelebolus globosus, Candida albicans* and *Melampsora epitea* were identified with a 100% identity match. All of them were detected with a low percent (1-3%) of the total findings amplified with this primers. The genus *Thelebolus* are known to grow on dung (De Hoog et al., 2005), whilst *Candida albicans* is a yeast species also found in birds (Cafarchia et al., 2008; Lord et al., 2010) and *Melampsora epitea* is a common parasite of arctic willows (Smith et al., 2004). In the top 20 list using the Bryo01 primers, multiple families, genera and species of mosses were detected with a 100% identity match. However, in the following I chose to focus only on the results from the G/H primer dataset, in order to look into where in the digestive system most of the total detected MOTUs appeared.

1.3.2 Results obtained with the G/H primers

From analysis of the negative controls, possible contamination between crop and gizzard samples were observed. After the removal of all rare and abundant sequences found in the negative controls, three negative controls were still showing reads. One control was taken from the mortars, another one taken from the liquid nitrogen and the last one was an empty tube staying open during the procedure. These controls were taken during the homogenization of the crop and gizzard samples with the use of ceramic mortar and liquid nitrogen. This means that the contamination could only occur for crop and gizzard samples, as other samples (both types of feces) were not grinded. Due to this possible contamination between crop and gizzard samples, I chose not to look at their results separately as I cannot for sure interpret differences between them. Therefore, I only used individuals with at least three different sample types (i.e. crop, gizzard and feces from the large intestine), which resulted in six

individuals with these three sample types and three individuals with four sample types (including feces from the ceca). All of these individuals were of the species willow ptarmigan.

The most abundant MOTU found in the the total list based on pooled results from different digestive organs belonged to the tribe Saliceae with a frequency of occurrence of approximately 67% (see appendix A5, Table A5.1). This MOTU was also the one with highest frequeny of occurence in the negative control dataset, with a percentage of almost 40% (see appendix A6, Table A6.1). The Saliceae MOTU also occurs in the fecal samples from the large intestine, which could not have been contaminated like the crops and gizzard may have.

All together 27 MOTUs were detected in the six individuals (Table 1.3) having at least three samples (representing crop, gizzard and fecal samples from the large intestine).

Table 1.3. The 27 diet MOTUs detected across the three sample types from the six individuals, with the most frequent MOTUs listed first. The most specific rank from either of the databases (i.e. EMBL or Ecochange) was used for the naming of the MOTUs. Abbreviations: ll = *Lagopus lagopus*, C = crop, G = gizzard and F = feces from the large intestine.

MOTUs	RANK	ll_001	ll_002	ll_011	ll_015	ll_017	ll_020
Saliceae	**Tribe**	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F
Vaccinium	**Genus**	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F
Empetrum	**Genus**	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F
Betulacea	**Family**	.-.-.	C-G-F	C-G-F	.-G-F	C-G-F	C-G-F
Dryadoideae	**Subfamily**	.-.-.	.-.-.	.-.-.	C-G-F	C-G-F	C-G-F
Vaccinium uliginosum	**Species**	.-.-.	.-.-F	C-G-F	C-G-F	.-.-.	C-G-F
Cerastium	**Genus**	.-.-.	.-G-.	.-.-.	.-.-.	.-.-.	.-.-.
Saxifraga oppositifolia	**Species**	.-.-.	C-G-.	.-.-.	.-.-.	.-.-.	.-.-.
Vaccinium boninense	**Species**	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F
Cochlearia	**Genus**	.-.-.	.-G-.	.-.-.	.-.-.	C-G-F	.-.-.
Saliceae	**Tribe**	C-G-F	.-.-F	C-G-F	C-G-F	C-G-F	C-G-F
Asteraceae	**Family**	.-.-.	C-.-.	.-.-.	C-G-F	.-.-.	.-G-.
Oxyria digyna	**Species**	.-.-.	.-.-F	.-.-.	.-.-.	.-.-.	.-.-.
Draba	**Genus**	.-.-.	.-.-.	.-.-.	.-G-.	.-.-.	.-.-.

Saxifragaceae	**Family**	.-.-.	.-.-.	.-.-.	.-G-.	.-.-.	.-.-.
Cardamine pratensis	**Species**	C-.-.	.-.-.	.-.-.	.-.-.	.-.-.	.-.-.
Vaccinium uliginosum	**Species**	.-.-.	.-.-.	C-G-F	C-G-F	.-.-.	C-G-F
Vaccinium vitis-idaea	**Species**	.-.-.	.-.-.	.-.-.	C-G-F	.-.-.	C-.-F
Asteraceae	**Family**	.-.-.	.-.-.	.-.-.	C-G-F	.-.-.	.-G-.
Calluna vulgaris	**Species**	.-.-.	.-.-.	.-.-.	.-G-.	.-G-.	.-.-.
Arctostaphylos uva-ursi	**Species**	.-.-.	.-.-.	.-.-F	.-.-.	.-.-.	.-G-F
Pooideae	**Subfamily**	.-.-.	.-.-.	.-.-.	.-.-.	.-.-.	C-.-.
Vaccinium uliginosum	**Species**	C-G-.	C-G-F	C-G-F	C-G-F	C-G-F	C-G-F
Alchemilla alpina	**Species**	.-.-.	.-.-.	.-.-.	.-G-.	.-.-.	.-.-.
Pyrola	**Genus**	.-.-.	.-.-.	.-.-.	.-.-F	.-.-.	.-.-.
Vaccinium vitis-idaea	**Species**	.-.-.	.-.-.	.-.-.	C-G-F	.-.-.	.-.-.
Saxifraga oppositifolia	**Species**	.-.-.	.-G-.	.-.-.	.-.-.	.-.-.	.-.-.

On overall, 68.9% of MOTUs listed above were detected in all three sample types. Table 1.4 shows the frequency of occurrence of a MOTU in the different sample types, either alone or in combinations. Using these numbers, the frequency of occurrence of a MOTU in feces from the large intestine was 79.7%, whilst the total diet detection rate in crop and gizzard combined was 93.2%.

Table 1.4. The table shows the frequency of occurrence of a MOTU in the different sample types, either alone or with other sample types in different combinations. Abbreviations: C = crop, G = gizzard and F = feces from the large intestine.

C-G-F	.-G-.	.-.-F	C-.-.	.-G-F	C-G-.	C-.-F
0.68918919	0.13513514	0.06756757	0.04054054	0.02702703	0.02702703	0.01351351

For the three individuals with four different sample types (representing crop, gizzard, feces from the large intestine and feces from the ceca), a total of 23 diet MOTUs (Table 1.5) were detected.

Table 1.5. The 23 diet MOTUs detected across the four sample types from the three individuals, with the most frequent MOTUs listed first. The most specific rank from either of the databases (i.e. EMBL or Ecochange) was used for the name of the MOTUs. Abbreviations: ll = *Lagopus lagopus*, C = crop, G = gizzard, F = feces from the large intestine, IC = feces from the ceca.

MOTUs	RANK	ll_001	ll_002	ll_011
Saliceae	Tribe	C-G-F-IC	C-G-F-IC	C-G-F-IC
Vaccinium	Genus	C-G-F-IC	C-G-F-IC	C-G-F-IC
Empetrum	Genus	C-G-F-IC	C-G-F-IC	C-G-F-IC
Betulaceae	Family	.-.-.-IC	C-G-F-IC	C-G-F-IC
Dryadoideae	Subfamily	.-.-.-.	.-.-.-.	.-.-.-IC
Vaccinium uliginosum	Species	.-.-.-.	.-.-F-.	C-G-F-IC
Cerastium	Genus	.-.-.-.	.-G-.-IC	.-.-.-.
Saxifraga oppositifolia	Species	.-.-.-.	C-G-.-.	.-.-.-.
Vaccinium boninense	Species	C-G-F-IC	C-G-F-IC	C-G-F-IC
Cochlearia	Genus	.-.-.-.	.-G-.	.-.-.-.
Saliceae	Tribe	C-G-F-IC	.-.-F-IC	C-G-F-IC
Asteraceae	Family	.-.-.-.	C-.-.IC	.-.-.-.
Oxyria digyna	Species	.-.-.-.	.-.-F-.	.-.-.-.
Cardamine pratensis	Species	C-.-.-.	.-.-.-.	.-.-.-.
Vaccinium uliginosum	Species	.-.-.-.	.-.-.-.	C-G-F-IC
Bistorta vivipara	Species	.-.-.IC	.-.-.-.	.-.-.-.
Vaccinium vitis-idaea	Species	.-.-.-.	.-.-.IC	.-.-.-.
Asteraceae	Family	.-.-.IC	.-.-.IC	.-.-.-.
Arctostaphylos uva-ursi	Species	.-.-.IC	.-.-.-.	.-.-F-IC
Vaccinium uliginosum	Species	C-G-.IC	C-G-F-IC	C-G-F-IC
Pyrola	Genus	.-.-.-.	.-.-.-IC	.-.-.-.
Vaccinium vitis-idaea	Species	.-.-.-.	.-.-.-IC	.-.-.-.
Saxifraga oppositifolia	Species	.-.-.-.	.-G-.-.	.-.-.-.

Table 1.6 show the frequency of occurrence of a MOTU in the four different sample types, either alone or in combinations. By including the additional type of feces (i.e. feces from the ceca), the frequency of occurrence of a MOTU in both feces increased to 90%, whilst 67.5%

of their diet was detected in the crop and gizzard samples combined. Ceca has also the highest detection rate of all the digestive organs, with 22.5% of the total MOTUs only detected in the ceca.

Table 1.6. The table shows the frequency of occurrence of a MOTU in the four different sample types, either alone or with other sample types in different combinations. Abbreviations: C = crop, G = gizzard, F = feces from the large intestine, IC = feces from the ceca.

C-G-F-IC	.-.-.-IC	.-.-F-.	.-.-F-IC	.-G-.-.	.-G-.-IC	C-G-.-IC	C-.-.-.	C-.-.-IC	C-G-.-.
0.500	0.225	0.050	0.050	0.050	0.025	0.025	0.025	0.025	0.025

By using the table above above and the *setdiff* function in R, results showed that 30% of the MOTUs detected in feces from the ceca were not detected in feces from the large intestine. At the opposite, only 5% of the MOTUs detected in feces from the large intestine were not detected in feces from the ceca.

1.4 Discussion

In this chapter I wanted to compare the MOTUs detected in samples taken from crop, gizzard, large intestine (fecal) and the ceca (fecal), and especially how much of the total MOTUs detected were identified in the fecal samples from the large intestine, and thereby answering the question: A) Are feces, especially feces from the large intestine, representative samples of the ptarmigan diet?

When combining the detection success across all sample types, counted as percentage of MOTUs, it is shown that including or excluding the feces from the ceca changes the overall result. When excluding the feces from the ceca, then crop and gizzard samples combined detected a larger proportion of the ptarmigan diet (crop and gizzard: 93.2%, fecal samples from large intestine: 79.7%). Whilst when including the feces from the ceca, the fecal samples combined (i.e. fecal samples from large intestine and fecal samples from ceca) detected a larger proportion of the ptarmigan diet than the crop and gizzard samples combined (crop and gizzard: 67.5%, fecal samples from large intestine and ceca: 90%). The explanation to why the two types of fecal samples combined detected more MOTUs may be due to the fact that contents in the ceca might stay longer, and become fermented in order to produce volatile fatty acids, especially because it is undigested nutrients that have bypassed the small intestine that reaches the ceca (Svihus et al., 2013). Therefore, MOTUs detected in feces from the ceca may illustrate a larger time frame of foraging, compared to the other digestive samples (i.e. crop, gizzard and feces from the large intestine). However, as ceca is absent in granivorous avian species (e.g. passerines) and well developed in omnivores (e.g. galliformes, which includes ptarmigan) and graminivores (e.g. geese), it has been suggested that the ceca play a role in breakdown of fibre in birds that sometimes feed on fibre-rich vegetable material (Svihus et al., 2013). Our results show that, as compared with the other digestive organs, ceca harbors the highest of unique MOTUs detected with DNA metabarcoding.

Oehm et al. (2011) discussed what may affect the detection success of prey DNA in avian (carrion crow) feces, and pointed out gut transition time as being an important factor, especially as this is poorly known. They also found that prey detection was possible from 5-day-old feces for birds fed with insect larvae. Pompanon et al. (2012) stated that the digestion process may differentially degrade the DNA from various dietary food items, and Rosenberg

et al. (1990) showed that these differential digestion rates can impose the largest bias in dietary studies using gut contents. To my knowledge, there have not been any avian studies comparing for how long known fed dietary food items are detactable in gut samples compared to fecal samples. Therefore, it is uncertain whether fecal samples are comparable with crop and gizzard samples when not taking into account for potential differential digestion rates. In addition, it is unknown whether the detected MOTUs originated from one or several meals, ingested over few days or longer time periods. Estimating transitition times for how long the different dietary MOTUs are expected to be detected is therefore an important knowledge, requiring further studies. In addition, it has been shown in birds that post-mortem digestion may occur, and therefore some researchers have injected formalin after death in order to stop the digestion prior diet analysis (Rosenberg et al., 1990, and references within). Another potential problem is that degraded DNA has shorter fragment lengths which in turn limits the number of fragments that can be successfully amplified (Pompanon et al., 2012). This means that if the digestion process differentially degrade the various dietary DNA, then theroretically some dietary food items may be underrepresented. This may also be the reason why the total diet detection was lower in feces from the large intestine compared to the combination of crop and gizzard, as the dietary food items found in crop and gizzard are less digested than fecal samples. However, it seems like when a MOTU is very abundant (i.e. high frequency of occurrence), like for example the MOTU belonging to the tribe Saliceae, it is detected in every sample type. Therefore, it is important to be critical and take into account what might bias the results. Despite the multiple potential biases, most of the diet diversity was captured using fecal samples from the large intestine.

1.5 Conclusion

My primary objective in this chapter was to test whether or not feces were representative samples of the ptarmigan diet. When including both type of feces, feces from the large intestine and feces from the ceca, the overall result was that the fecal samples detected a higher percentage of the diet (90%), than samples from crop and gizzard combined (67.5%). However, this result does not necessarily take into account whether the detected MOTUs originated from a single meal or from multiple meals over several days. As dietary items could still be potentially detectable up to at least five days in avian fecal samples, the potential ingestion of several meals might introduce bias in the results. When excluding the fecal samples from the ceca, approximately 13% fewer dietary food items were detected from the fecal samples from the large intestine (79.7%) than from the crop and gizzard samples combined (93.2%). However, despite the decrease, I will conclude that fecal samples from the large intestine are good representative sample types for dietary analysis in ptarmigans, and eventual other herbivorous birds with similar digestive system, as most of the diet diversity was captured. For other organisms the results may be different. Despite this, DNA metabarcoding of fecal samples from the large intestine appear as a valuable tool for diet analysis, allowing to capture large proportion of diet taxonomical diversity with identifications often at the genus- or species-level.

2 Trophic interactions between herbivore species in Finnmark

2.1 Introduction

In ecology theory, a central component is that coexisting species use different resources, i.e. use resource partitioning to avoid competition (Behmer and Joern, 2008). In the Arctic tundra, the resources (i.e. potential diet foods) may be limited because less than 1% of the world total of vascular plants and about 6% of the world total of bryophytes are found in the Arctic (Meltofte, 2013). This can hypothetically lead to the sharing (i.e. a niche overlap) of critical resources. Soininen et al. (2015) found that there is actually a high diet overlap between herbivores in the High Arctic, but the food resources may be abundant enough for the herbivores to cope.

The Arctic tundra is home of numereous herbivores. By studying how the resources are allocated between different herbivores helps to understand their roles and interactions, and is important due to the rapid changes observed in the ecosystems around the world (Tylianakis et al., 2008, and references within). Global change drivers can influence species coexistence and interactions, and there is an urgent need to understand the direction of the changes (Valladares et al., 2015). It is therefore important to understand the trophic interactions between the herbivores and their potential niche overlap. In addition to how much their diet might overlap, it is also important to understand what their diets consists of.

In this chapter I have analysed the diets of five taxonomic group of herbivores; ptarmigan, reindeer, hare, rodent and moose. To my knowledge, never have such divergent taxonomic group of herbivores been analysed for potential niche overlaps, except between African large herbivores (Kartzinel et al., 2015). However, there are many studies on diet overlap between different ungulates in the northern climates, e.g. wild reindeer and domestic sheep in Norway (Skogland, 1984): moose, roe deer, red deer, sheep, goat and reindeer in Fennoscandia (Mysterud, 2000): roe deer, red deer, chamois and moufloin in northern French Alps (Redjadj et al., 2014), as well as between different rodent species in northern Norway (Soininen et al., 2014: Soininen et al., 2015).

The results gained from this chapter will not only give us better understanding of the five Norwegian herbivores in Finnmark and their trophic interactions, but also the possibility to integrate this information into the developing of potential future sustainable management and conservation plans which may be needed due to effects of further global warming. With the use of DNA metabarcoding of field-collected feces from the sites where herbivore species co-exist, I will in this chapter answer the following question: B) Is there a dietary niche overlap among the herbivore species in the Norwegian arctic tundra, and how high is this potential overlap?

2.2 Material and methods

2.2.1 Study sites

My sampling took place near Alta in Western Finnmark, Norway, on the territory of a regional reindeer herding area in district 22. My supervisors had already established two sampling locations (site 1 and site 2) with approximately 5 km between (Fig. 2.1). These sites were representing the typical landscape features of grazing areas, with one site on a steeper slope (S2) compared to the other site (S1).

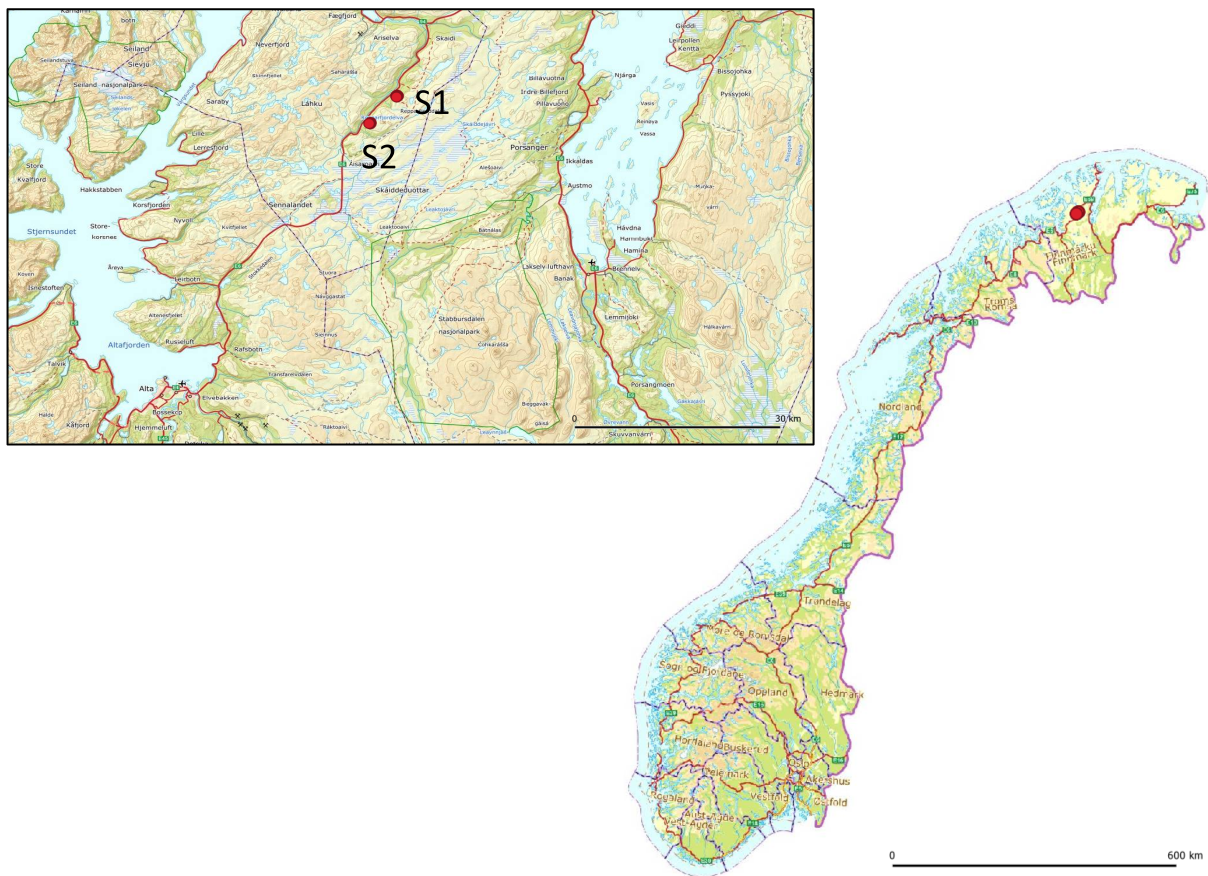


Figure 2.1. Overview of the sampling locations (site 1 and site 2) in northern Norway. The sampling locations are located near Alta, in Western Finnmark. Maps were made in QGIS v.3.4 (https://qgis.org), by downloading the map package "Matrikkel Bagrunn2" from Geonorge (https://www.geonorge.no).

Above the tree-limit (Fig. 2.2 a), where some of the samples were collected, the landscape consisted of mountain tundra. Whilst below the tree-limit (Fig. 2.2 b), the samples were sampled within a birch forest with undergrowth including shrubs, semishrubs, graminoids, mosses, ferns and lichens.

(a)

(b)

Figure 2.2. Overview of the two main arctic tundra habitats, with (a) showing above the tree-limit with mountain tundra, and (b) showing below the tree-limit in a birch forest. Pictures taken during the sampling.

2.2.2 Sample collection and handling

At each location, a set of four (site 2) or five (site 1) sampling quadrates (50 x 50 cm) were placed along an altitudinal gradient encompassing the two main arctic tundra habitats in the region (Fig. 2.3). Each gradient was replicated three times at each location, with a 100 m distance between the plots. Care was taken by placing the plots in places with obvious activities of rodents, such as holes.

(a) (b)

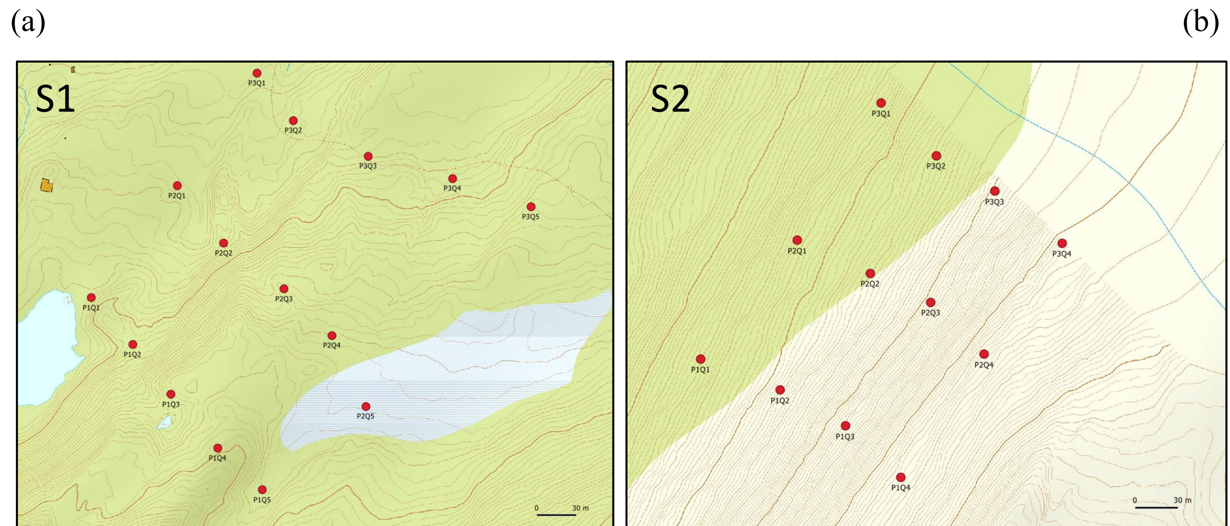


Figure 2.3. The pictures show how the plots were distributed at site 1 (a) and site 2 (b), along an altitudinal gradient. The gradient was replicated three times at both locations, with a set of four sampling quadrates at site 1 and five sampling quadrates at site 2. Maps were made in QGIS v.3.4 (https://qgis.org), by downloading the map package "Matrikkel Bagrunn2" from Geonorge (https://www.geonorge.no).

In July 2017, a minimum of five and maximum of 10 rodent feces (i.e. the number of feces that was available) were collected within each plot, and plots were thereafter entirely cleaned from the remaining feces. Additionally, all available reindeer, moose, hare and ptarmigan fecal samples were collected in immediate proximity to each plot. In early September using the same approach, I collected rodent and other herbivore fecal samples from the same plots. The collected fecal samples were preserved in vials pre-filled with silica gel and stored at -20°C at the University of Oslo.

2.2.3 Molecular diet analysis

2.2.3.1 Sample preparation and DNA extractions

All samples were left to thaw at room temperatures for a few hours before they were homogenized by hand-crushing into a fine powder between two sheets of aluminum foil. Between <100 and 250 mg of homogenized powder were then subsampled according to the quantity of material available and the degree of water content (i.e. if the sample was dry we aimed for 100 mg if available, and if the sample was wet we took 250 mg). Sub-samples were taken using disposable spatulas (Chemglass, UK) and transferred into new sterile 2-ml microcentrifuge tubes. Subsampled material was stored at -20°C prior to molecular diet analysis. Sterile, empty micro-centrifuge tubes were held open during sample homogenization in order to monitor for possible cross-contaminations by powder particles.

Total DNA from all subsamples was extracted in random order using the DNeasy PowerSoil kit (Qiagen) according to manufacturer's instructions. DNA was eluted in a total volume of 100 µl and stored at -20°C. Negative controls (no DNA) were systematically included in order to monitor for potential cross-contamination.

2.2.3.2 PCR amplification and high-throughput sequencing

Procedures for PCR amplifications, gel-electrophoresis, purifications of amplicons and preparations for high-througput sequencing are strictly the same as in Chapter 1, with the following exceptions:

- Samples were divided on two PCR plates.
- Every PCR reaction was replicated three times.
- We generated eight pools after purification.

The eight pools were pooled again in equimolar proportions prior library preparations and sequencing. This final pooling resulted in four final pool contents at the end, where products from each PCR plate were divided into two tubes (one of these pools contained all the PCR products amplified with the vascular plant and bryophytes primers, and the second pool contained all the PCR products amplified with the eukaryote and fungi primers).

2.2.4 Bioinformatic analyses

Bioinformatic analyses were carried out as described in Chapter 1. Number of raw sequences produced per library is given in Table 2.2.

Table 2.2. Overview of the libraries and their content after the receiving of the raw sequences from the Norwegian Sequencing Center.

Library ProcessID	Library content	Number of raw sequences
Lib6	GH+BRYO Mix	16 375 713
Lib7	GH+BRYO Finnmark	17 045 113
Lib13	EUKA+FUNGI Finnmark	15 966 649
Lib14	EUKA+FUNGI Mix	15 987 205

2.2.5 Data filtering and statistical analyses

The data filtering and statistical analyses were done in R, R software v.3.3.2 (R Core Team, 2016), using RStudio. Results for each of the primers were filtered and analysed separately. Unfortunately, one out of the two PCR plates had to be discarded due to that its three PCR replicates were not similar. After discarding the outliers on this plate, approximately half of the samples were lost, while the remaining samples had mostly only one out of three replicates left. With only one out of two PCR plates left, the samples were not analysed with focus on eventual differences between the two main altitudinal gradients, nor to find out if there were seasonal differences between the diets sampled in July (i.e. winter and spring diet) and September (summer diet).

G/H dataset

G/H tabulated files based on taxonomic assignations from EMBL and Ecochange reference databases were imported and analysed together using ROBITools package. First step of filtering consisted of the removal of all MOTUs flagged as PCR errors, which were the MOTUs that appeared with "internal" status. Next step was the removal of all MOTUs with below 65% identity match (i.e. the match of a sequence to the reference database) in order to not eliminate too many real sequences, with the EMBL database, as well as the removal of MOTUs represented with less than 1% of the total number of reads in a PCR (i.e. rare MOTUs). This resulted in the majority of sequences having a 100% match with the EMBL database, and only a few appeared below 95% match.

All samples were subdivided into different categories (e.g. ”Herbivores”, “Ptarmigan”, “Reindeer”, “Rodent”, “Hare”, “Moose” and ”Controls”), in order to easily extract the sample information needed during the analyses. With the use of data plotting software ggplot together with tidyverse and ggplot2 packages, I visualized the number of reads and MOTUs in each category. The category “Controls” containing all types of controls, was further investigated and showed low numbers of both reads and MOTU´s. Four MOTU´s (see appendix B1, Table B1.1) were detected as being more frequent (counts between 96 and 158) in the negative controls compared to the experimental samples, and were therefore removed entirely from the dataset. The positive controls were detected as expected, thus confirming that the PCR reactions were successful.

Next step consisted of looking into the PCR replicates. The first inspection showed mostly none or low distances between the corresponding replicates. All outliers were discarded from the dataset, and only highly similar PCR replicates were retained. The remaining replicates were then merged together. The resulting dataset contained 70 herbivore samples, of which 25 were ptarmigan samples, 2 were reindeer samples, 8 were moose samples, 9 were hare samples and 26 were rodent samples.

All of the 70 herbivore samples were compared with the use of multivariate analyses. The output sequences (128 MOTUs) were Hellinger-transformed (using the vegan package) in order to run a Principal Component Analysis (PCA) (using the ade4 package). PCA is used to reduce dimensionality and study variation in data matrix by transforming the original variables into linear combinations to generate the axes called Principal Components (PCs). This technique will make the data easy to visualize, as the first and second PCs used are the ones which account for highest variance in the data. The PCA plots helped in order to see ordination of all the samples, and how they might relate to each other in terms of diet.

In addition, boxplots were made with the use of different Hill numbers (Hill, 1973) (with the use of vegan package). Three diversity indices were calculated; richness ($q=0$), Shannon index ($q=1$) and Gini index ($q=2$), where parameter q determines the sensitivity of a diversity index to the relative abundances.

Pie charts were made for each of the herbivores separately, as well as one pie chart for all of the herbivores combined. The pies were calculated using the frequency of each MOTU being detected in the diets. In order to make the pie charts, all MOTUs found with the G/H primers

were divided into seven categories ("Shrub", "Betula" "Tree", "Forb", "Graminoid", "Moss", "Fern" and "Other"). Appendix B2 (Table B2.1-B2.8) shows which categories all of the 125 MOTUs were divided into. Grasses and sedges were put together in the category "Graminoid". Herbs and forbs that are neither a grass or a sedge were put together in the category "Forb". Everything that did not fit into "Shrub", "Betula", "Tree", "Forb", "Graminoid", "Moss" or "Fern" were put in the category "Other".

Euka02 dataset

The same procedure was done for this dataset. The majority of the sequences were within 96-100% best match with the EMBL database, but with many appearing down to 65% match. The threshold for identity match was therefore set to 90%.

The category "Controls" showed higher number of reads and MOTUs than for the G/H dataset. Thirteen MOTUs (see appendix B1, Table B1.2) were detected as being more frequent (counts between 112 and 269575) in the negative controls compared to the experimental samples, and were therefore removed entirely from the dataset.

The PCR replicates in this dataset showed none or low distances between the corresponding replicates. Therefore, the same procedure was done as in the G/H dataset. The resulting dataset contained 70 herbivore samples, of which 24 were ptarmigan samples, 2 were reindeer samples, 8 were moose samples, 9 were hare samples and 27 were rodent samples.

The number of output sequences (172 MOTUs) containing all herbivore samples, were Hellinger-transformed in order to make a Principal Component Analysis (PCA).

Fung01 dataset

The same procedure was done for this dataset. However, the sequences were spread between 65% and 100% best match with the EMBL, even though more sequences had a 100% match. The threshold for identity match was therefore set to 90%.

Eleven MOTUs (see appendix B1, Table B1.3) were detected as being more frequent (counts between 5 and 1759) in the negative controls compared to the experimental samples, and were therefore removed entirely from the dataset.

The PCR replicates in this dataset showed mostly none or low distances between the corresponding replicates. Therefore, the same procedure was done as in the G/H dataset. The

resulting dataset contained 69 herbivore samples, of which 24 were ptarmigan samples, 1 was reindeer sample, 8 were moose samples, 9 were hare samples and 27 were rodent samples.

The number of output sequences (194 MOTUs) containing all herbivore samples, were Hellinger transformed in order to make a Principal Component Analysis (PCA).

Bryo01 dataset

The same procedure was done for this dataset. The majority of the sequences had a 100% best match with the EMBL database, and only a few appeared below 95% match.

Low number of reads and MOTUs were detected in the "Control" category, and none appeared as being more frequent in the negative controls compared to the experimental samples. Therefore none MOTUs were discarded in this step.

The PCR replicates in this dataset showed mostly none or low distances between the corresponding replicates. Therefore, the same procedure was done as in the G/H dataset. The resulting dataset contained 68 herbivore samples, of which 23 were ptarmigan samples, 1 was reindeer sample, 8 were moose samples, 9 were hare samples and 27 were rodent samples.

The number of output sequences (94 MOTUs) containing all herbivore samples, were Hellinger transformed in order to make a Principal Component Analysis (PCA).

2.3 Results

2.3.1 Comparison of results obtained with the four primer sets

Comparison of the ordination plots (PCAs) of herbivore diet composition based on the four different metabarcoding primers (Fig. 2.4), showed that Fung01 primers provided more clear grouping with less overlap between the herbivore species, while the other primers provided more overlap with only some tendency of species specific diet.

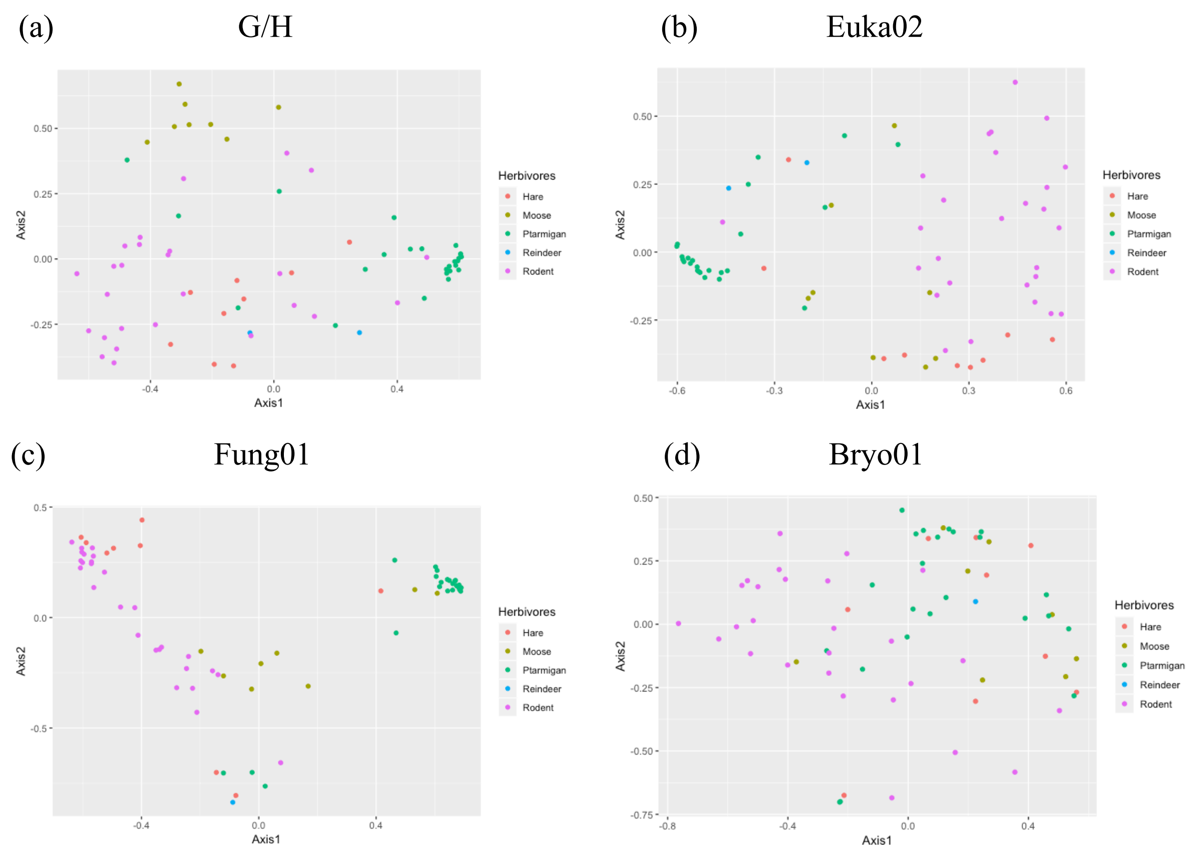


Figure 2.4. PCA on diet variation of the five herbivores with the use of (a) G/H primers, (b) Euka02 primers, (c) Fung01 primers and (d) Bryo01 primers. The different herbivores are color coded. Each dot represents one sample.

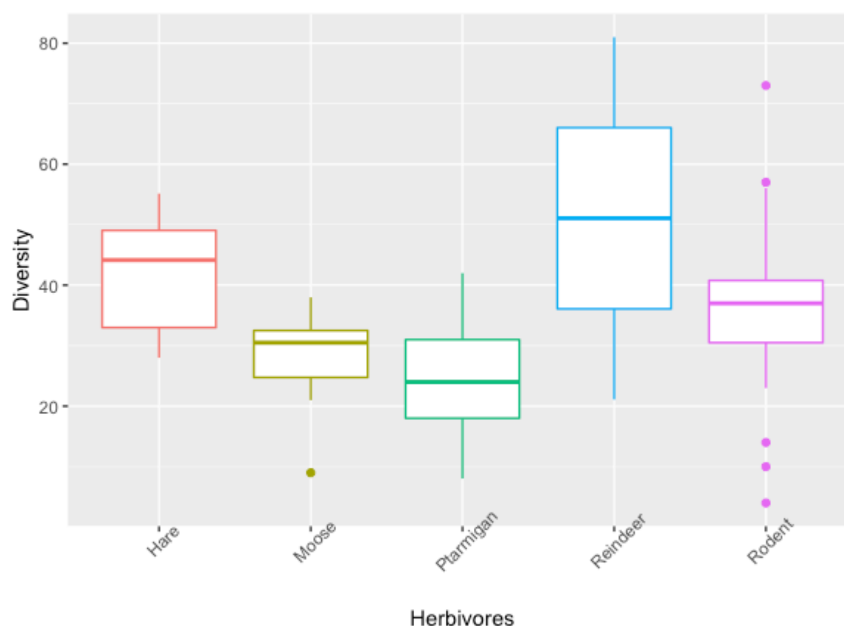
Top 20 lists of the most frequent MOTUs found with each of the primers for the herbivores combined are presented in appendix B3, Table B3.1-B3.4. The G/H primers detected different kind of shrubs, graminoids, forbs, ferns, mosses and trees. The Euka02 primers did not detect any other potential diet food that were not already detected with the G/H primers. The Fung01 primers mainly detected fungal microorganisms, plant pathogens and fungi that grow on feces. Thereby, it seems that the detected fungi were not a direct source of food eaten by the

herbivores, meaning that the PCA on Fig. 2.4(c) does not reflect any diet related grouping. The Bryo01 primers detected different kinds of mosses and liverworths, which were also detected with the G/H primers. Only the findings detected with the G/H primers were therefore further used.

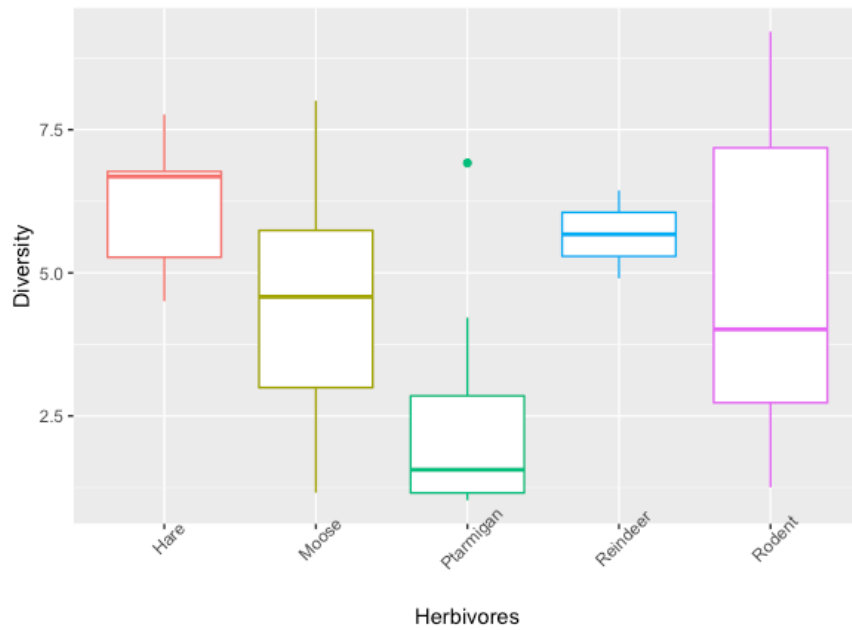
2.3.2 Results obtained with the G/H primers

In order to look into the herbivores' diet diversity, three boxplot analyses were performed (Fig. 2.5). In the first analysis (a), richness was represented by the number of unique MOTUs detected in herbivores feces (q = 0). In the second analysis (b), diversity was represented by the use of Shannon index (q = 1). This means that rare species with low abundances were suppressed in the boxplot. In the third analysis (c), diversity was represented with the use of Gini index (q = 2), which strengthens the signals from the Shannon index, meaning that the abundant species contributed more while the rare species contributed less. The boxplot analyses showed that the range of species richness values as well as average species richness seems to be the highest in reindeer, and the lowest for ptarmigan, as compared to the rest of the species (Fig. 2.5a). The dispersion of diversity values increased in moose and rodents, and decreased for reindeer, when applying Shannon (Fig. 2.5b) and Gini (Fig. 2.5c) indices. The values for ptarmigan were still low when applying Shannon and Gini indicec.

(a) Richness



b) Shannon index



(c) Gini index

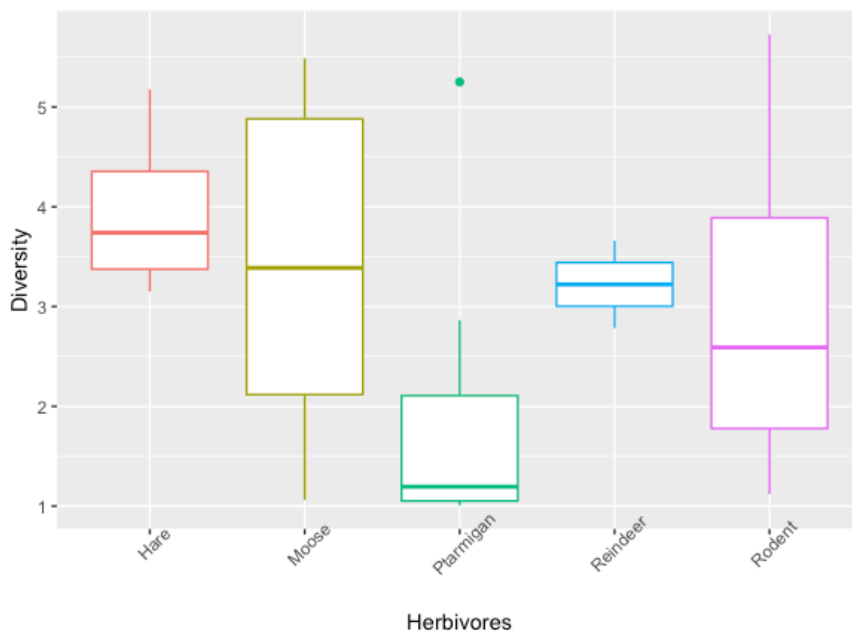
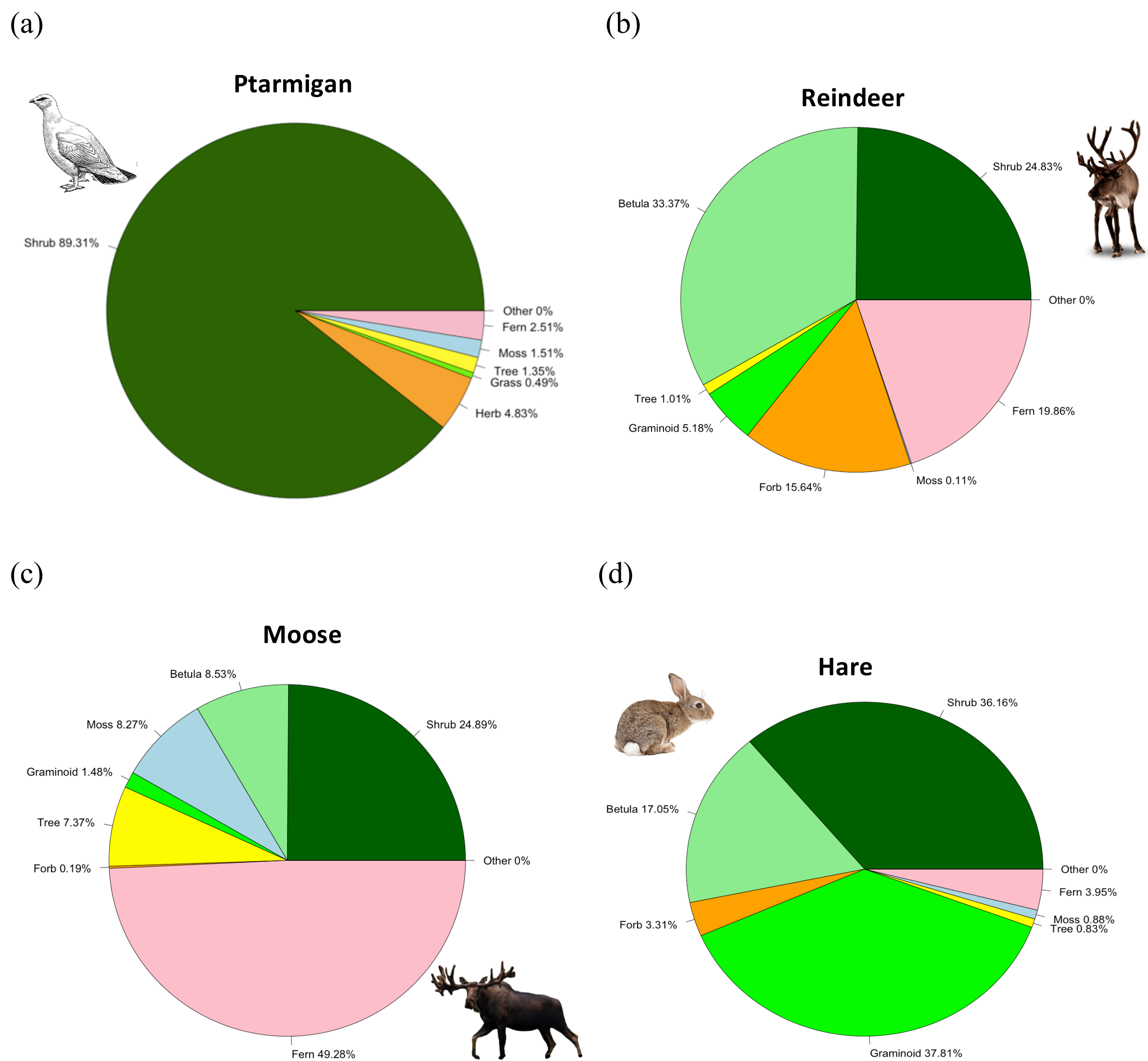
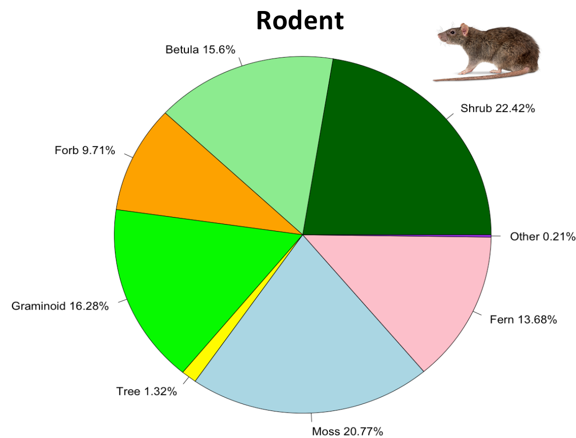


Figure 2.5. Boxplots showing diversity of diet taxa on y-axis, and type of herbivores on x-axis. (a) shows the diversity as richness in numbers of unique MOTUs detected in diet, (b) shows the diet diversity with the use of Shannon index and, (c) shows the diet diversity with the use of Gini index. Lower and upper limits of each box represent respectively 25% and 75% quartiles. Thickened lines within each box represent the sample median. Lines above and below each box (whiskers) represent the minimum and maximum values. Dots represent outliers.

The pie charts (Fig. 2.6) reval that the five taxonomic group of herbivores seem to have different preferences with regard to relative amounts of the seven dietary categories. Most of the ptarmigan diet (Fig.2.6a) consisted of *Betula* (73.15%) and shrubs (15.34%). The reindeer diet (Fig. 2.6a) consisted mainly of *Betula* (33.37%) and shrubs (24.83%), but also some ferns (19.86%) and forbs (15.64%). Almost half of the moose diet (Fig. 2.6c) consisted of ferns (49.28%), whereas shrubs made up approximately a quarter of their diet (24.89%). The hare diet (Fig. 2.6d), mainly consisted of shrubs (36.16%) and graminoids (37.81%), together with *Betula* (17.05%). For the rodents (Fig. 2.6e) it is shown a more even distribution of the different dietary cateogies, but still shrubs were detected with the highest frequency (22.42%). When looking at the combined diet of the five herbivores (Fig. 2.6f), we see that *Betula* was the most shared category, followed by shrubs.



(e)



(f)

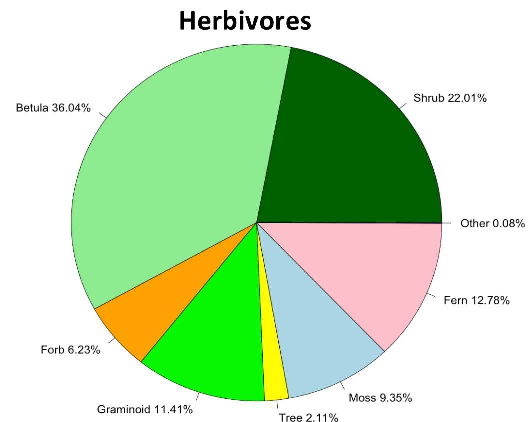


Figure 2.6. Pie charts made from sequences amplified by the G/H primers showing each of the herbivore diets divided into seven categories ("Shrub", "Betula", "Tree", "Forb", "Graminoid", "Moss", "Fern" and "Other"), where (a) shows the ptarmigan diet, (b) shows the reindeer diet, (c) shows the moose diet, (d) shows the hare diet, (e) shows the rodent diet and (f) shows the combined dataset of all of the herbivores.
Pictures: Ptarmigan, reindeer, moose, hare, rodent downloaded from www.uihere.com, with all pictures free for any use.

The lists with the 20 most abundant taxa detected in the herbivores feces (see appendix B4, Table B4.1-B4.5) revealed that *Betula* was the most common and shared diet taxon based on sequences amplified with G/H primers. All of the herbivores had *Betula* as their most eaten diet taxon, except for moose, which had *Betula* as its fourth most eaten diet taxon. *Betula* maked up 73.15% of the ptarmigan's diet, 33.37% of the reindeer's diet, 8.53% of the moose's diet, 17.05% of the hare's diet and 15.6% of the rodent's diet. It seems that ptarmigans could be more dependent on *Betula* as one of their main diet taxa compared to the other herbivores, which had much lower frequencies of *Betula* in their diet.

Based on these results, a food web (Fig. 2.7) was manually constructed with information from the lists with the top 20 most abundant taxa for each of the herbivore species. Only shrubs and *Betula* MOTUs appearing with a frequency above 1% in each herbivore species diet were extracted in order to make the food web. Within shrubs, four dietary items were detected with frequency of occurrence above 1%. The shrub genus *Empetrum* was detected from ptarmigan and rodent feces. The shrub species *Vaccinium ulignosum* and genus *Vaccinium* were detected from ptarmigan, reindeer, hare and rodent feces. While the shrub tribe Saliceae was detected from ptarmigan, hare and moose feces.

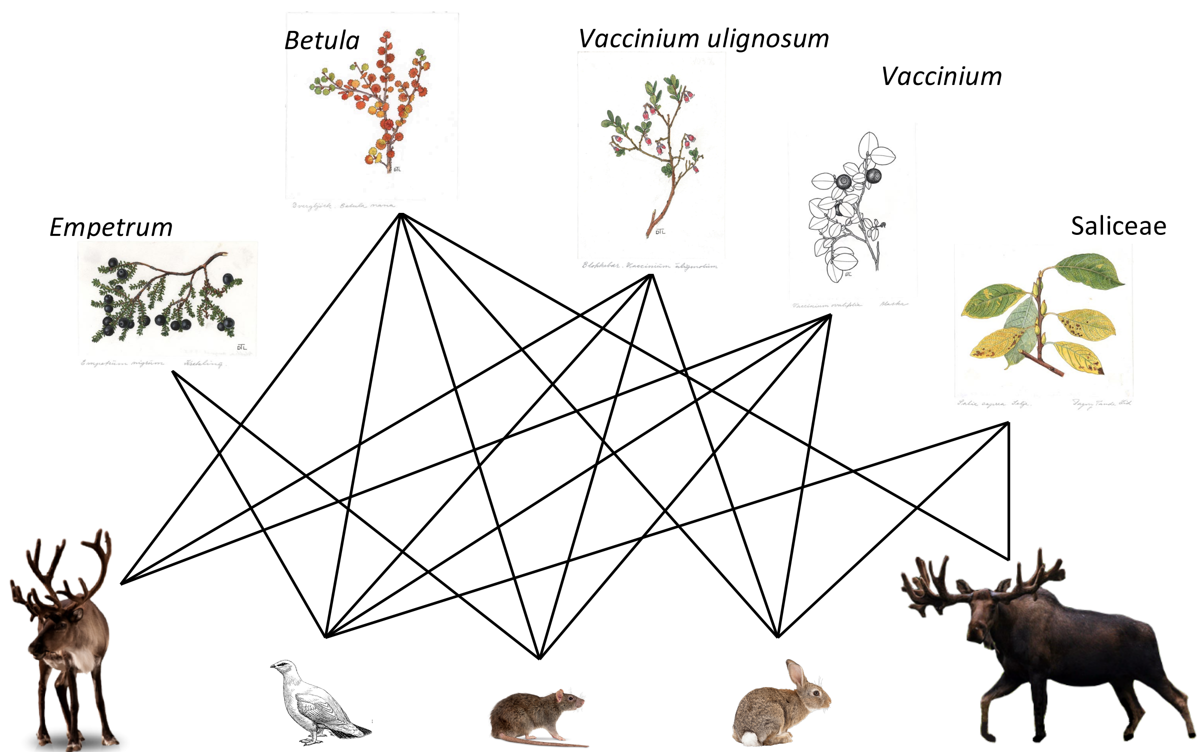


Figure 2.7. Food web constructed manually based on the lists with the top 20 most abundant taxa retrieved for each of the herbivore species, based on sequences detected with the G/H primers. Only shrubs and *Betula* MOTUs appearing with a frequency above 1% in each herbivore species' diet were extracted in order to make the food web.
Pictures: Reindeer, ptarmigan, rodent, hare and moose downloaded from www.uihere.com, with all pictures free for any use. *Betula*, *Vaccinium* spp., *Empetrum* and Saliceae downloaded from Dagny Tande Lids botanic drawings, https://www.nhm.uio.no/fakta/botanikk/karplanter/dagny-tande-lid/tegningene/, with the usage of the pictures covered by BONO agreement.

2.4 Discussion

In this study, the main focus was to detect potential niche overlaps between the five common herbivore species inhabiting Finnmark (i.e. ptarmigan, reindeer, hare, rodent and moose), and thereby answering the question: B) Is there a dietary niche overlap among the herbivore species in the Norwegian arctic tundra, and how is this potential overlap?

Based on all pie chart results (Fig. 2.6), variation in diet composition were observed between species, but also that they seemed to be mostly browsers meaning that their diet largely consisted of shrubs. All of the studied herbivores in this chapter had high frequencies of *Betula* in their diet, but *Betula* was only categorized down to genus which mean it can theoretically be either a shrub (e.g. *Betula nana*) or a tree (e.g. *Betula pubescens*). Therefore, we can not confirm from these results whether there is a niche overlap regarding *Betula*, or whether the herbivores have fed on different species within the same genus. In addition, as *Betula* is windpollinated and produces large amounts of pollen, it is possible that contaminations might have occurred, either out in the field or at a later stage as most of the labwork was carried out during summer. Another potential challenge with the use of only DNA metabarcoding for diet analyis, is that we could not know, for the same plant taxon, whether different herbivores consumed exactly the same part of that taxon. For example, theoretically some herbivores might only feed on the berries of a plant, while other herbivores would prefere the stem or the leaves of the same plant. Therefore, food resource partitioning might exists among herbivores in order to allow them to coexist, as discussed by Kartzinel et al. (2015).

Reindeer were expected to have been feeding on lichen, as lichen is known to dominate the reindeer diet in winter (Heggberget et al., 2002), and is also preferred to other food items (Kojola et al., 1995, and references within). However, lichen was not found with any of the primers. It could be that lichens were overgrazed during the time of the forage, which in turn should increase the proportions of vascular plants and mosses in their diet (Kojola et al, 1995, and references within). On the other hand, mosses were almost not detected (0.11%) in the reindeer diet with the use of G/H primers, but *Betula*, shrubs, ferns and forbs were all detected with frequencies above 15%. For ptarmigan, the results showed high correspondence with results from published data on crop content of willow ptarmigan in Finland. In particular, *Betula pubescens* together with *Salix* sp. were reported as the main components of winter diet

(Pullainen and Iivanainen, 1981). Based on the boxplot analyses (Fig. 2.5), using Shannon (Fig. 2.5b) and Gini indices (Fig. 2.5b), ptarmigan seemed to show the lowest dietary diversity, which is also shown in the ptarmigan's pie chart (Fig. 2.6a) that their diet consisted mainly of *Betula* and shrubs.

Based on my results, it is not possible to conclude that the MOTUs relative frequencies actually represent the ingested biomass, therefore my results rather provide an overview of the occurrences of output sequences and their detectable frequencies. We can therefore only assume that a MOTU appearing in many individuals (versus MOTUs appearing in few individuals) seems to be frequently consumed. Pompanon et al. (2012, and references within) stated that the PCR amplification process may lead to a bias in the representation of ingested food proportions. This may be due to for example primer mismatches, resulting in some sequences becoming under-represented, even though they still may be important and abundant diet taxa. This might theroretically have been the case to why lichens were not detected in the reindeer fecal samples. In addition, if the DNA in dietary samples is degraded, this may limit the length of the fragments that can be successfully amplified. Kartzinel et al. (2015) also stated that DNA metabarcoding has not been used that much in studies testing hypotheses about niche relationships among species, and therefore few studies have cross-validated results gained from different DNA-based approaches about quantitative consumption.

The results might also be biased to a certain degree due to the fact that mammal-specific primers were not used. Therefore, the field-collected fecal samples from the studied herbivore species may have been mis-identified as belonging to the wrong species. On the other hand, as the herbivores feces look different, especially regarding their size, such bias is not very likely in my case. Also, we do not know how old the collected feces were, thus if the feces represent winter, spring, summer or autumn diet. The only exception are for the rodent feces collected in September 2017, which should at maximum be approximately two months old. Feces which have been laying out in the field for a longer time, might be contaminated by the environment. The samples could also represent multiple species, as well as multiple individuals, especially regarding the rodent feces. This is due to the fact that more feces were needed to obtain as close as possible to 100mg, which needed for each DNA extraction. Also, because there are two ptarmigan species in Norway, the ptarmigan diet might have been biased as we did not know to which species the ptarmigan samples belong, meaning that their inferred diet could be a combination of the two species.

Unfortunately, a lot of samples did not make it to the results. One possible explanation for why the three replicate in one of the PCR plates, containing approximately half of the herbivore samples from Finnmark, showed low similarity, could be low DNA quality (due to e.g. degradation or non-optimal conservation of the samples) resulting in the replicates not replicating correctly. However, this is most likely not the case as in the other PCR plate the three replicates showed high similarity. It is more likely that something went wrong during the lab preparations, before sending the samples for sequencing. Because of this I was not able to analyse the herbivore diets related to the two main altitudinal gradients (i.e. above and below the tree-limit), nor to compare for eventual seasonal differences in the herbivore diets, as inititally planned.

Despite multiple potential biases, it is still observed clear differences among the five different taxonomic group of herbivores diet and their proportions of MOTUs. Thus, meaning DNA metabarcoding of field-collected fecal samples seem to be a useful tool for niche overlap analyses.

2.5 Conclusion

My primary objective in this chapter was to find out if there is a dietary niche overlap between the five studied herbivore species (i.e. ptarmigan, reindeer, rodent, hare and moose) in Norwegian arctic habitats. Despite a limited number of samples analysed, interesting patterns were observed, especially regaring the distribution of shrubs and *Betula*, across the herbivores.

Almost three quarters of the ptarmigan diet consisted of *Betula*, suggesting that ptarmigans seem to be the species relying most on *Betula* as its main dietary food item. On the contrary, moose seem to be the species that relies the least on *Betula*, with detection of *Betula* being less than 10%. *Betula* or shrubs dominated ptarmigan, reindeer, hare and rodent diet, while ferns followed by shrubs dominated the moose diet. The tribe Saliceae was the only shared shrub in moose and two of the other herbivores (ptarmigan and hare) diet. The dwarf shrub genus *Empetrum* was shared in ptarmigan and rodent diet. The dwarf shrub species *Vaccinium ulignosum* and genus *Vaccinium* were shared in ptarmigan, reindeer, hare and rodent diet. Based on my results, there might be a possibility for competition among the species. Especially ptarmigans could hypothetically be more vulnerable to both competition for food resources and changes in the plant community, which may happen due to increasing surface air temperatures. However, in order to find out out if ptarmigans and the other four herbivores do eat the same part of the shared diet taxa, DNA metabarcoding could be combined with for example direct observation in future studies. It is also important to note that the results obtained in this chapter did not take into account possible seasonal variations of the herbivores' diets. However, by successfully using field-collected fecal samples, multiple shared MOTUs between the five taxonomic group of herbivores were detected. This indicate that a high niche overlap exist, but to a lesser extent when including moose.

General conclusion

The results from Chapter 1 showed when including both type of feces, feces from the large intestine and feces from the ceca, the overall results were that the fecal samples detected a higher percentage of the total detected diet (90%), than samples from crop and gizzard combined (67.5%). When excluding the fecal samples from the ceca, fecal samples from the large intestine still detected 79.7% of the diet compared to 93.2 % in crop and gizzard combined. In conclusion, if it is not necessary to detect recently consumed food items (e.g. from last meal), as dietary food items are detectable ut to at least five days in avian fecal samples, fecal samples from the large intestine are still a good representative sample type for dietary analysis using DNA metabarcoding, as it allows to capture most of the diet diversity. However, this conclusion is based on observations of the digestive system of ptarmigans, meaning that results may be different for other herbivorous species. Despite this, DNA metabarcoding of fecal samples from the large intestine appear as a valuable tool for diet analysis, allowing to capture large proportion of diet taxonomical diversity with identifications often at the genus- or the species-level. Therefore, in Chapter 2, I applied the same method on field-collected fecal samples from five different taxonomic groups of herbivores to investigate if there is a dietary niche overlap among them in the Norwegian arctic tundra. By successfully using the field-collected fecal samples, multiple shared MOTUs between the herbivores were detected. These results indicate that high niche overlap exist between the studied herbivore species, but to a lesser extent when including moose. Altogether, the results from both chapters bring new knowledge about herbivores assemblage in the Arctic, and provide methodological developments for enhanced characterization of arctic food webs.

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Appendix A – Comparing digestive samples from ptarmigan using DNA metabarcoding

A1. Ptarmigan samples

Table A1.1. Overview of all ptarmigan specimens, shot date, location and what type of samples were sampled. Abbreviations: lmh = *Lagopus muta hyperborea*. lm = *Lagopus muta*. ll = *Lagopus lagopus*.

Table A1.1. The table shows all of the ptarmigan samples, their sample name, shot date, shot location and the types of sampled collected from each individual.

Sample name	Shot date	Shot location	Crop	Gizzard	Large Intestine	Ceca
lmh_003	Oct. 2015	Akseløya (Svalbard)		Yes	Yes	
lmh_004	Oct.2015	Akseløya (Svalbard)		Yes	Yes	
lmh_005	Oct. 2015	Akseløya (Svalbard)		Yes	Yes	
lmh_006	Oct.2015	Akseløya (Svalbard)		Yes	Yes	
lmh_007	Oct. 2015	Akseløya (Svalbard)		Yes	Yes	
lmh_008	Oct. 2015	Akseløya (Svalbard)		Yes	Yes	
lmh_009	Oct. 2015	Akseløya (Svalbard)		Yes	Yes	
lmh_010	Oct. 2015	Akseløya (Svalbard)		Yes	Yes	
lm_b	Oct/Nov 2015	Tromsø		Yes	Yes	
lm_c	29.Oct.2016	Tromsø		Yes	Yes	
lm_001	15.Oct. – 23.Dec. 2014?	Kvaløya (Tromsø)			Yes	
lm_002	11.Oct.2015	Tromsø			Yes	
lm_003	11.Oct.2015	Tromsø			Yes	
lm_004	29.Oct.2016	Tromsø			Yes	
lm_005	Oct/Nov 2015	Tromsø			Yes	
lm_010	Oct/Nov 2015	Tromsø			Yes	
ll_001	Oct/Nov 2015	Tromsø	Yes	Yes	Yes	Yes
ll_002	Oct/Nov 2015	Tromsø	Yes	Yes	Yes	Yes
ll_005	Oct/Nov 2015	Tromsø		Yes	Yes	Yes
ll_009	Oct/Nov 2015	Tromsø		Yes	Yes	Yes
ll_010	Oct/Nov 2015	Tromsø		Yes	Yes	Yes
ll_011	Oct/Nov 2015	Tromsø	Yes	Yes	Yes	Yes
ll_012	Oct/Nov 2015	Tromsø		Yes	Yes	Yes
ll_015	Oct/Nov 2015	Tromsø	Yes	Yes	Yes	Yes
ll_019	Oct/Nov 2015	Tromsø		Yes	Yes	Yes
ll_014	Oct/Nov 2015	Tromsø	Yes	Yes	Yes	
ll_016	15.Oct. – 23.Dec. 2014?	Kvaløya (tromsø)		Yes		
ll_017	Oct/Nov 2015	Tromsø	Yes	Yes	Yes	
ll_018	Oct/Nov 2015	Tromsø	Yes	Yes	Yes	
ll_020	Oct/Nov 2015	Tromsø	Yes	Yes	Yes	
ll_022	29.Nov.2016	Tromsø	Yes	Yes	Yes	

A2. Manipulated ptarmigans

Table A2.1 – A2.2. Overview all ptarmigans in this thesis, with eventual information about previous manipulating of the specimens before my arrival at Jennifer Forbey´s lab. Sample names in *italic* are found in both tables.

Table A2.1. Overview of all manipulations done with the ptarmigans in this thesis before my arrival at Jennifer Forbey´s lab. The specimens marked in colors are manipulated twice (see Table A2.2). Abbreviations: JF = Jennifer Forbey. lmh = *Lagopus muta hyperborea*. lm = *Lagopus muta.* ll = *Lagopus lagopus*.

Sample name	Notes
lmh_003	JF took crop contents and liver sample. Kept rest of guts in tact.
lmh_004	JF took crop contents and liver sample. Kept rest of guts in tact.
lmh_005	JF took crop contents and liver sample. Kept rest of guts in tact.
lmh_006	JF took crop contents and liver sample. Kept rest of guts in tact.
lmh_007	JF took crop contents and liver sample. Kept rest of guts in tact.
lmh_008	JF took crop contents and liver sample. Kept rest of guts in tact.
lmh_009	JF took crop contents and liver sample. Kept rest of guts in tact.
lmh_010	JF took crop contents and liver sample. Kept rest of guts in tact.
lm_b	JF took samples from liver, proximal and distal small intestine, large intestine and ceca.
lm_c	No information.
lm_001	JF took liver sample and removed the crop and gizzard. Kept rest of guts in tact.
lm_002	JF took liver sample and removed the crop and gizzard. Kept rest of guts in tact.
lm_003	JF took liver sample and removed the crop and gizzard. Kept rest of guts in tact.
lm_004	JF took liver sample and removed the crop and gizzard. Kept rest of guts in tact.
lm_005	JF took liver sample and removed the crop and gizzard. Kept rest of guts in tact.
lm_010	JF took liver sample and removed the crop and gizzard. Kept rest of guts in tact.
ll_001	JF took liver samples. Kept rest of guts in tact.
ll_002	JF took liver samples. Kept rest of guts in tact.
ll_005	JF took liver sample and removed the crop. Kept rest of guts in tact.
ll_009	JF took liver sample and removed the crop. Kept rest of guts in tact.
ll_010	JF took liver sample and removed the crop. Kept rest of guts in tact.
ll_011	JF took liver samples. Kept rest of guts in tact.
ll_012	JF took liver samples. Kept rest of guts in tact.
ll_015	JF took liver samples. Kept rest of guts in tact.
ll_019	JF took liver sample and removed the crop. Kept rest of guts in tact.
ll_014	JF took liver samples and cut of the crop without taking it. Kept rest of guts in tact.
ll_016	JF took liver samples and cut of the crop without taking it. Kept rest of guts in tact.
ll_017	JF took liver samples. Kept rest of guts in tact.

ll_018	JF took liver samples. Kept rest of guts in tact.
ll_020	JF took liver samples. Kept rest of guts in tact.
ll_022	JF took liver samples. Kept rest of guts in tact.

Table A2.2. Showing the 14 specimens manipulated by an undergraduate at Jennifer Forbey´s lab, before my arrival, in order to measure digestive track lengths. Crop and gizzard were untouched, whilst the large intestine may possible have been contaminated from the environment.

Lagopus lagopus	***Lagopus muta***	***Lagopus muta hyperborea***
ll_014	*lm_001*	*lmh_003*
ll_016	*lm_002*	*lmh_004*
ll_017	*lm_003*	*lmh_005*
ll_018		*lmh_008*
ll_020		*lmh_010*
ll_022		

A3. Cycling conditions

Table A3.1 - A3.4. Overview of the cycling conditions used to run PCR with the different primers (i.e. G/H, Euka02, Fung01 and Bryo01).

Table A3.1. The table shows the cycling conditions used to run PCR with the G/H primers, for detection of vascular plants.

PCR conditions: G/H		
Initial denaturation	95°C	10 mn
Nb cycles	**35**	
Denaturation	95°C	30 s
Hybridation	52°C	30 s
Elongation	72°C	1 mn
Final elongation	72°C	7 mn
Hold	15°C	forever

Table A3.2. The table shows the cycling conditions used to run PCR with the Euka02 primers, for detection of eukaryotes.

PCR conditions: Euka02		
Initial denaturation	95°C	10 mn
Nb cycles	**35**	
Denaturation	95°C	30 s
Hybridation	45°C	30 s
Elongation	72°C	1 mn
Final elongation	72°C	7 mn
Hold	15°C	forever

Table A3.3. The table shows the cycling conditions used to run PCR with the Fung01 primers, for detection of fungi.

PCR conditions: Fung01		
Initial denaturation	95°C	10 mn
Nb cycles	**35**	
Denaturation	95°C	30 s
Hybridation	56°C	30 s
Elongation	72°C	1 mn
Final elongation	72°C	7 mn
Hold	15°C	forever

Table A3.4. The table shows the cycling conditions used to run PCR with the Bryo01 primers, for detection of bryophytes.

PCR conditions: Bryo01		
Initial denaturation	95°C	10 mn
Nb cycles	**35**	
Denaturation	95°C	30 s
Hybridation	54°C	30 s
Elongation	72°C	1 mn
Final elongation	72°C	7 mn
Hold:	15°C	forever

A4. MOTUs detected in negative control dataset with each primer

Table A4.1 – A4.3. Overview of the MOTUs detected as being more frequent in negative control datasets compared to the experimental samples. They were removed from the datasets.

Table A4.1. Overview of the 12 MOTUs detected as being more frequent in negative controls compared to the experimentam samples, with the G/H primers.

MOTUs	Scientific name (EMBL)	Count
P_GH_00000417	Solanoideae	9333
P_GH_00004111	Gossypium	5127
P_GH_00002086	Pooideae	2547
P_GH_00001120	Allium	703
P_GH_00079916	Allium sativum	536
P_GH_00054559	Allium	517
P_GH_00023620	Prunus	473
P_GH_00007886	Cyamopsis tetragonoloba	446
P_GH_00054308	Musaceae	317
P_GH_00005015	Capsicum	301
P_GH_00017334	PACMAD clade	118
P_GH_00102164	Vaccinium vitis-idaea	47

Table A4.2. Overview of the top 10 out of 73 MOTUs with highest counts detected as being more frequent in negative controls compared to the experimentam samples, with the Euka02 primers.

MOTUs	Scientific name (EMBL)	Count
P_EU_00000285	rosids	9779
P_EU_00000467	Zea mays	5739
P_EU_00000320	Cordyceps memorabilis	5484
P_EU_00002668	Pezizomycotina	2551
P_EU_00002043	Silene vulgaris	2230
P_EU_00003007	Penicillium	1472
P_EU_00008682	Malvoideae	1278
P_EU_00002578	Debaryomycetaceae	844
P_EU_00037515	Plectosphaerellaceae	815
P_EU_00005697	Alternaria	781

Table A4.3. Overview of the 11 MOTUs detected as being more frequent in negative controls compared to the experimentam samples, with the Fung01 primers.

MOTUs	Scientific name (EMBL)	Count
P_FU_00001845	Penicillium	1119
P_FU_00005392	Eukaryota	794
P_FU_00008129	Nigrospora	783
P_FU_00002335	Fusarium	712
P_FU_00001497	Aspergillus penicillioides	676
P_FU_00010271	Colletotrichum	304
P_FU_00054048	Gibellulopsis	230
P_FU_00048393	Vanrija nantouana	169
P_FU_00019797	Malassezia	147
P_FU_00013845	Corynespora	100
P_FU_00038833	Leptosphaeria sp. RJ-2015	6

A5. Top 20 MOTUs detected in ptarmigans with each primer

Table A5.1 – A5.4. Overview of the top 20 most frequent MOTUs detected in the ptarmigan datasets with each primer.

Table A5.1. Overview of the top 20 most frequent MOTUs detected in the ptarmigan dataset with the G/H primers.

MOTUs	Frequency	Scientific name (Ecochange)	Best identity (Ecochange)	Scientific name (EMBL)	Best identity (EMBL)
P_GH_00000001	0.6713331214	Saliceae	1.0000000	Saliceae	1.0000000
P_GH_00000017	0.1200650966	*Vaccinium*	1.0000000	Vaccinieae	1.0000000
P_GH_00000022	0.0770284183	*Empetrum*	1.0000000	*Empetrum*	1.0000000
P_GH_00000037	0.0334610120	*Betula*	1.0000000	Betulaceae	1.0000000
P_GH_00000038	0.0246267755	*Vaccinium uliginosum*	1.0000000	*Vaccinium*	1.0000000
P_GH_00000471	0.0159871331	*Cerastium*	1.0000000	Alsineae	1.0000000
P_GH_00000008	0.0116050191	*Saxifraga oppositifolia*	1.0000000	*Saxifraga*	1.0000000
P_GH_00000328	0.0112025488	*Vaccinium*	0.9791667	*Vaccinium boninense*	1.0000000
P_GH_00000157	0.0060740514	Maleae	1.0000000	Maleae	0.9803922
P_GH_00000011	0.0045559700	*Dryas*	1.0000000	Dryadoideae	1.0000000
P_GH_00000019	0.0035212288	*Cochlearia*	1.0000000	*Cochlearia*	1.0000000
P_GH_00001045	0.0020133969	*Oxyria digyna*	1.0000000	*Oxyria digyna*	1.0000000
P_GH_00000382	0.0019914579	Gnaphalieae	1.0000000	Asteraceae	0.9800000
P_GH_00000585	0.0015149225	*Cardamine pratensis*	1.0000000	*Cardamine*	1.0000000
P_GH_00000490	0.0015027050	Saxifragaceae	1.0000000	Saxifragaceae	1.0000000
P_GH_00001786	0.0013253650	*Saxifraga cespitosa*	1.0000000	*Saxifraga*	1.0000000
P_GH_00000158	0.0011638289	*Draba*	1.0000000	Arabideae	1.0000000
P_GH_00000338	0.0011441532	*Saxifraga*	0.9666667	*Saxifraga*	1.0000000
P_GH_00006582	0.0006978072	*Festuca*	1.0000000	Pooideae	1.0000000
P_GH_00001444	0.0006763818	*Stellaria*	1.0000000	*Stellaria*	1.0000000

Table A5.2. Overview of the top 20 most frequent MOTUs detected in the ptarmigan dataset with the Euka02 primers.

Motus	Frequency	Scientific name (EMBL)	Rank	Best Identity
P_EU_00000007	0.513025272	*Gallus gallus*	Species	0.9902913
P_EU_00000004	0.259299240	Malpighiales	Order	0.9901961
P_EU_00000001	0.084136629	*Vaccinium macrocarpon*	Species	1.0000000
P_EU_00000098	0.023456549	Ericales	Order	0.9901961
P_EU_00000054	0.015013671	*Betula*	Genus	1.0000000
P_EU_00000160	0.012434076	Fungi	Kingdom	0.9900000
P_EU_00000010	0.010218841	*Cerastium arcticum*	Species	1.0000000
P_EU_00001011	0.009286560	*Clupea harengus*	Species	1.0000000
P_EU_00000066	0.006482127	Saxifragales	Order	0.9902913
P_EU_00000056	0.005176466	Boreoeutheria	No rank	0.9904762
P_EU_00000070	0.004979231	eudicotyledons	No rank	0.9711538
P_EU_00000702	0.004763303	Passifloraceae	Family	0.9901961
P_EU_00000163	0.004161102	leotiomyceta	No rank	0.9898990
P_EU_00001401	0.003826433	*Rheum*	Genus	0.9803922
P_EU_00000058	0.003352726	Theaceae	Family	1.0000000
P_EU_00002706	0.003090438	*Cladosporium sp. CB-20*	Species	0.9900000
P_EU_00000401	0.002765666	Eukaryota	Superkingdom	0.7500000
P_EU_00000002	0.002628826	Haloragaceae	Family	0.9803922
P_EU_00003234	0.002366480	Eukaryota	Superkingdom	0.7500000
P_EU_00000133	0.002340247	Ericaceae	Family	0.9901961

Table A5.3. Overview of the top 20 most frequent MOTUs detected in the ptarmigan dataset with the fung01 primers.

MOTUs	Frequency	Scientific name (EMBL)	Rank	Best Identity
P_FU_00000062	0.039366015	Heliotiales	Order	0.8855721
P_FU_00000018	0.032202216	*Thelebolus globosus*	Species	1.0000000
P_FU_00000013	0.028939004	*Candida albicans*	Species	0.9942197
P_FU_00000071	0.028557748	*Claussenomyces sp.*	Species	0.9497487
P_FU_00000012	0.027774688	*Thelebolus*	Genus	1.0000000
P_FU_00000193	0.023218971	*Gremmeniella*	Genus	0.9418605
P_FU_00000138	0.021033383	Eukaryota	Superkingdom	0.8443396
P_FU_00000232	0.017927159	Leotiomycetes	Class	0.8663366
P_FU_00000014	0.016575876	*Candida albicans*	Species	1.0000000
P_FU_00000148	0.016032465	*Taphrina*	Genus	0.7682403
P_FU_00014158	0.015003007	*Malassezia restricta*	Species	0.9877049
P_FU_00000350	0.014381046	*Phacidiopycnis sp.*	Species	0.9948718
P_FU_00026673	0.014084507	Eukaryota	Superkingdom	0.9122137
P_FU_00000003	0.013500472	Leptosphaeria sp. RJ-2015	Species	0.9622642
P_FU_00000050	0.013244983	*Mrakia*	Genus	0.8356808
P_FU_00000185	0.012356530	*Phaetromella*	Genus	0.9185185
P_FU_00001202	0.012057292	*Venturia*	Genus	0.9740933
P_FU_00000047	0.011862084	*Herpotrichia juniperi*	Species	0.9880240
P_FU_00000383	0.010238416	*Melampsora epitea*	Species	1.0000000
P_FU_00000108	0.009875341	Leotiomycetes	Class	0.8939394

Table A5.4. Overview of the top 20 most frequent MOTUs detected in the ptarmigan dataset with the bryo01 primers.

MOTUs	**Frequency**	**Scientific name (EMBL)**	**Rank**	**Best Identity**
P_BR_00000027	0.16791858	*Kiaeria*	Genus	1.0000000
P_BR_00000002	0.16150322	*Sanionia*	Genus	1.0000000
P_BR_00000068	0.10158461	Hypnales	Order	0.9811321
P_BR_00000006	0.09002961	*Dicranum*	Genus	1.0000000
P_BR_00000132	0.03377133	*Pseudoleskea patens*	Species	1.0000000
P_BR_00000011	0.02974577	*Pohlia nutans*	Species	0.9787234
P_BR_00000012	0.02377780	Hypnales	Order	0.9811321
P_BR_00000001	0.02360570	Polytrichaceae	Family	1.0000000
P_BR_00000126	0.02124959	*Hamatocaulis*	Genus	0.9811321
P_BR_00000637	0.02087215	Grimmiaceae	Family	1.0000000
P_BR_00000030	0.02049455	*Distichium capillaceum*	Species	1.0000000
P_BR_00006445	0.02047486	Polytrichaceae	Family	0.9795918
P_BR_00000107	0.02017473	Hypnales	Order	0.9811321
P_BR_00000029	0.01926971	Bryaceae	Family	1.0000000
P_BR_00000115	0.01836557	*Plagiomnium*	Genus	1.0000000
P_BR_00000053	0.01641667	*Rhizomnium gracile*	Species	1.0000000
P_BR_00000033	0.01599000	*Drepanocladus arcticus*	Species	0.9830508
P_BR_00000117	0.01590394	*Cryphaeae tasmanica*	Species	0.9814815
P_BR_00000016	0.01246275	Hypnales	Order	0.9811321
P_BR_00000123	0.0109357	Pottiaceae	Family	1.0000000

A6. Top 20 MOTUs detected in controls after removal of frequent rare sequences

Table A6.1. Overview of the top 20 most frequent MOTUs detected in the control dataset after the removal of the rare frequent sequences that appeared in the controls, with the G/H primers.

Table A6.1. Overview of the top 20 most frequent MOTUs detected in the controls with the G/H primers, after the removal of all the frequent rare sequences that appearead in the controls. These controls illustrates the results coming from three different negative controls (mortar, liquid nitrogen and an open tube during the grinding with liquid nitrogen in mortars).

MOTUs	Frequency	Scientific name (Ecochange)
P_GH_00000001	0.3971275822	Saliceae
P_GH_00000037	0.1419010812	Betulaceae
P_GH_00000017	0.1001820265	*Vaccinium*
P_GH_00000471	0.0321814280	*Cerastium*
P_GH_00000008	0.0196733888	*Saxifraga oppositifolia*
P_GH_00000022	0.0195320161	*Empetrum*
P_GH_00000328	0.0192959326	*Vaccinium boninense*
P_GH_00000019	0.0147769483	*Cochlearia*
P_GH_00001786	0.0047091249	*Saxifraga cespitosa*
P_GH_00005180	0.0040073929	*Saxifraga*
P_GH_00008139	0.0026182001	*Saxifraga*
P_GH_00001299	0.0017398406	*Vaccinium vitis-idaea*
P_GH_00000457	0.0014775471	*Saxifraga oppositifolia*
P_GH_00008123	0.0009160355	*Saxifraga*
P_GH_00010487	0.0006747682	*Saxifraga*
P_GH_00000652	0.0006335650	*Vaccinium vitis-idaea*
P_GH_00000093	0.0005259186	Saliceae
P_GH_00004847	0.0005174858	Saxifragaceae
P_GH_00001500	0.0002123103	*Vaccinium uliginosum*
P_GH_00000038	0.0001287601	*Vaccinium uloginosum*

Appendix B – Trophic interactions between herbivore species in Finnmark

B1. MOTUs detected in negative control dataset with each primer

Table B1.1 – B1.3. Overview of the MOTUs detected as being more frequent in negative control datasets compared to the experimental samples. They were removed from the datasets.

Table B1.1. Overview of the 4 MOTUs detected as being more frequent in negative controls compared to the experimentam samples, with the G/H primers.

MOTUs	Scientific name (EMBL)	Count
F_GH_00043336	Pyrus	158
F_GH_00069920	Prunus	143
F_GH_00340578	Linum	130
F_GH_00130728	Arachis hypogaea	96

Table B1.2. Overview of the 13 MOTUs detected as being more frequent in negative controls compared to the experimentam samples, with the Euka02 primers.

MOTUs	Scientific name (EMBL)	Count
F_EU_00000141	Boreoeutheria	269575
F_EU_00000120	Salmonidae	15735
F_EU_00013306	Mesenchytraeus	1755
F_EU_00018570	Boreoeutheria	752
F_EU_00004405	Vigna angularis var. angularis	566
F_EU_00016616	Isotricha	470
F_EU_00018654	Powellomycetaceae	376
F_EU_00014362	Rhizophydiales	290
F_EU_00033278	Eukaryota	184
F_EU_00048718	Leptopharynx bromelicola	182
F_EU_00061881	Fungi	169
F_EU_00031719	Ustilago	134
F_EU_00037198	Leptosphaeria maculans species complex	112

Table B1.3. Overview of the 11 MOTUs detected as being more frequent in negative controls compared to the experimentam samples, with the Fung01 primers.

MOTUs	Scientific name (EMBL)	Count
F_FU_00008595	Preussia	1759
F_FU_00007225	Claussenomyces sp. PDD 95741	323
F_FU_00434583	cf. Trechispora sp. 2 LF-2015	59
F_FU_00003063	Hannaella coprosmae	50
F_FU_00278932	Fibricium subceraceum	48
F_FU_00055318	Debaryomyces	43
F_FU_00333839	Cladophialophora bantiana	37
F_FU_00269731	Aspergillus	34
F_FU_00318765	Cladosporium sphaerospermum	25
F_FU_00401503	Cladosporium	22
F_FU_01647691	Malassezia restricta	5

B2. Diet categories

Table B2.1 - B2.8. Overview of which categories all the detected MOTUs with the G/H primer were placed (i.e. ”Graminoid”, ”Shrub”, ”Betula”, ”Tree”, ”Forb”, ”Moss”, ”Fern” or ”Other”).

Table B2.1. Overview of the MOTUs detected with the G/H primers that were placed in the category ”Graminoid”.

Graminoid		
MOTUs	**Scientific name (Ecochange)**	**Scientific name (EMBL)**
F_GH_00000019	Pooideae	Poeae
F_GH_00000020	*Avenella flexuosa*	Poeae Chloroplast Group 2 (Poeae type)
F_GH_00000262	*Alopecurinae*	Poeae Chloroplast Group 2 (Poeae type)
F_GH_00000279	*Eriophorum*	Eriophorum
F_GH_00000284	Poeae	Poeae
F_GH_00000312	*Juncus trifidus*	*Oreojuncus*
F_GH_00000449	*Carex*	*Carex*
F_GH_00000525	*Pooideae*	*Poaceae*
F_GH_00000873	*Poeae*	*Poeae*
F_GH_00001504	*Luzula*	*Luzula*
F_GH_00001530	*Carex vaginata*	*Carex vaginata*
F_GH_00001622	Agrostidinae	Poeae Chloroplast Group 1 (Aveneae type)
F_GH_00002316	Carex lachenalii	Carex lachenalii
F_GH_00002890	Poeae	Pooideae
F_GH_00002955	Carex	Carex
F_GH_00004359	Puccinellia	Coleanthinae
F_GH_00005116	Luzula	Luzula
F_GH_00005992	Carex	Carex
F_GH_00011208	Poeae	Poeae Chloroplast Group 2 (Poeae type)
F_GH_00012614	*Luzula parviflora*	*Luzula*
F_GH_00020701	Poeae	*Avena*
F_GH_00003167	Festuca	*Pooideae*

Table B2.2. Overview of the MOTUs detected with the G/H primers that were placed in the category ”Shrub”.

	Shrub	
MOTUs	**Scientific name (Ecochange)**	**Scientific name (EMBL)**
F_GH_00000002	Saliceae	Saliceae
F_GH_00000008	*Vaccinium*	Vaccinieae
F_GH_00000010	*Dryas*	Dryadoideae
F_GH_00000029	*Vaccinium uliginosum*	*Vaccinium*
F_GH_00000044	*Vaccinium*	*Vaccinium boninense*
F_GH_00000071	*Empetrum*	*Empetrum*
F_GH_00000162	*Phyllodoce caerulea*	*Phyllodoce caerulea*
F_GH_00000289	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*
F_GH_00000340	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*
F_GH_00000806	*Empetrum*	Ericoideae
F_GH_00000935	*Phyllodoce caerulea*	*Phyllodoce caerulea*
F_GH_00000965	*Vaccinium vitis-idaea*	Vaccinioideae
F_GH_00001551	*Arctous*	*Arctostaphylos uva-ursi*
F_GH_00001652	*Andromeda polifolia*	Vaccinioideae
F_GH_00001810	*Vaccinium vitis-idaea*	Vaccinium vitis-idaea
F_GH_00002123	*Vaccinium vitis-idaea*	Vaccinium vitis-idaea
F_GH_00002235	Saliceae	Salicaceae
F_GH_00003374	*Kalmia procumbens*	*Kalmia procumbens*
F_GH_00004603	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*
F_GH_00004730	*Diapensia lapponica*	*Diapensia lapponica*
F_GH_00005771	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*
F_GH_00005840	*Vaccinium vitis-idaea*	*Vaccinioideae*
F_GH_00005847	*Vaccinium vitis-idaea*	*Ericaceae*
F_GH_00006350	*Linnaea borealis*	*Caprifoliaceae*
F_GH_00006966	*Kalmia procumbens*	*Kalmia procumbens*
F_GH_00008271	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*
F_GH_00009211	*Vaccinium vitis-idaea*	*Vaccinium vitis-idaea*

F_GH_00013009	*Arctous*	*Arctous*
F_GH_00015774	*Vaccinium vitis-idaea*	Vaccinioideae
F_GH_00016094	*Vaccinium vitis-idaea*	Vaccinioideae
F_GH_00150679	*Vaccinium vitis-idaea*	Vaccinioideae

Table B2.3. Overview of the MOTUs detected with the G/H primers that were placed in the category ”Betula”.

	Betula	
MOTUs	**Scientific name (Ecochange)**	**Scientific name (EMBL)**
F_GH_00000001	*Betula*	Betulaceae
F_GH_00000191	*Betula*	Betulaceae
F_GH_00001414	*Betula*	Betulaceae
F_GH_00696251	*Betula*	Fagales

Table B2.4. Overview of the MOTUs detected with the G/H primers that were placed in the category ”Tree”.

	Tree	
MOTUs	**Scientific name (Ecochange)**	**Scientific name (EMBL)**
F_GH_00000223	Maleae	Maleae
F_GH_00000259	*Pinus*	*Pinus*
F_GH_00000816	*Pinus*	*Pinus*
F_GH_00004013	*Juniperus*	Cupressaceae
F_GH_00043336	Maleae	*Pyrus*
F_GH_00069920	*Prunus*	*Prunus*

Table B2.5. Overview of the MOTUs detected with the G/H primers that were placed in the category ”Forb”.

	Forb	
MOTUs	**Scientific name (Ecochange)**	**Scientific name (EMBL)**
F_GH_00000041	*Cerastium fontanum*	*Cerastium*
F_GH_00000113	*Listera cordata*	*Neottia cordata*
F_GH_00000125	*Sagina*	Sagineae
F_GH_00000137	*Bistorta vivipara*	Polygonoideae

F_GH_00000138	*Saxifraga oppositifolia*	*Saxifraga*
F_GH_00000175	*Rumex*	Rumiceae
F_GH_00000267	*Cerastium*	Alsineae
F_GH_00000471	Asteroideae	Asteroideae
F_GH_00000562	Rosoideae	Rosaceae
F_GH_00001012	*Stellaria*	*Stellaria*
F_GH_00001167	Asteraceae	Asterales
F_GH_00001261	*Cochlearia*	*Cochlearia*
F_GH_00001419	*Trientalis*	*Trientalis*
F_GH_00001461	*Draba*	Arabideae
F_GH_00001478	*Saxifraga*	*Saxifraga*
F_GH_00001712	*Cornus suecica*	*Cornus suecica*
F_GH_00001765	*Cornus suecica*	*Cornus suecica*
F_GH_00002842	*Caltha*	*Caltha*
F_GH_00002923	*Ranunculus*	*Ranunculus*
F_GH_00003793	*Micranthes stellaris*	*Micranthes stellaris*
F_GH_00005082	*Geranium sylvaticum*	*Geranium*
F_GH_00005256	*Trollius*	Ranunculoideae
F_GH_00005518	*Potentilla*	*Potentilla*
F_GH_00005523	*Epilobium*	Epilobieae
F_GH_00006993	*Sagina*	Sagineae
F_GH_00009595	*Comarum palustre*	Potentilleae
F_GH_00012178	Apiaceae	Apioideae
F_GH_00014099	*Draba*	*Draba*
F_GH_00028561	*Viola*	*Viola brevistipulata*
F_GH_00059782	Saxifragaceae	Cucurbitaceae
F_GH_00068818	*Cakile*	Brassicaceae
F_GH_00096196	*Alchemilla*	*Alchemilla*
F_GH_00061942	*Cerastium cerastoides*	*Cerastium cerastoides*

F_GH_00340578	*Linum*	*Linum*

Table B2.6. Overview of the MOTUs detected with the G/H primers that were placed in the category ”Moss”.

Moss		
MOTUs	**Scientific name (Ecochange)**	**Scientific name (EMBL)**
F_GH_00000006	Polytrichaceae	Polytrichaceae
F_GH_00000022	*Sciuro-hypnum*	Brachytheciaceae
F_GH_00000187	Hypnales	Hypnales
F_GH_00000257	*Sanionia uncinata*	*Sanionia*
F_GH_00000451	*Dicranum*	Dicranaceae
F_GH_00000600	Lycopodiaceae	Lycopodioideae
F_GH_00000618	*Dicranum*	*Dicranum*
F_GH_00000974	Dicranaceae	Dicranidae
F_GH_00002144	*Sphagnum russowii*	*Sphagnum*
F_GH_00002239	Dicranaceae	Bryophytina
F_GH_00002553	*Bryum*	Bryaceae
F_GH_00003792	*Plagiothecium denticulatum*	Bryidae
F_GH_00013561	Eukaryota	Bryidae
F_GH_00020022	*Buxbaumia aphylla*	*Buxbaumia*
F_GH_00031902	*Leptobryum pyriforme*	*Leptobryum pyriforme*
F_GH_00032092	*Leptobryum pyriforme*	*Leptobryum pyriforme*
F_GH_00034111	*Pohlia*	*Splachnum luteum*
F_GH_00078869	*Tetraplodon pallidus*	*Tetraplodon pallidus*

Table B2.7. Overview of the MOTUs detected with the G/H primers that were placed in the category ”Fern”.

Fern		
MOTUs	**Scientific name (Ecochange)**	**Scientific name (EMBL)**
F_GH_00000047	Polypodiales	*Athyrium*
F_GH_00000073	*Gymnocarpium dryopteris*	*Gymnocarpium dryopteris*
F_GH_00000110	*Dryopteris*	*Dryopteris*
F_GH_00000553	*Phegopteris connectilis*	Polypodiales
F_GH_00000727	*Thelypteris palustris*	Polypodiales
F_GH_00000821	Polypodiales	*Athyrium sinense*
F_GH_00003467	*Equisetum*	*Equisetum*

Table B2.8. Overview of the MOTUs detected with the G/H primers that were placed in the category ”Other”.

Other		
MOTUs	**Scientific name (Ecochange)**	**Scientific name (EMBL)**
F_GH_00009823	Magnoliophyta	Soja
F_GH_00018211	Magnoliophyta	Poaceae
F_GH_00130728	Magnoliophyta	*Arachis hypogaea*

B3. Top 20 MOTUs detected in herbivore datasets with each primer

Table B3.1 – B3.4. Overview of the top 20 most frequent MOTUs detected in the herbivore datasets with each primer. Abbreviations: C. = Chloroplast.

Table B3.1. Overview of the top 20 most frequent MOTUs detected with the G/H primers.

MOTUs	Frequency	Scientific name (Ecochange)	Best identity (Ecochange)	Scientific name (EMBL)	Best identity (EMBL)
F_GH_00000001	0.359270332	*Betula*	1.0000000	Betulaceae	1.0000000
F_GH_00000047	0.086596013	Polypodiales	0.9487179	*Athyrium*	1.0000000
F_GH_00000020	0.069331687	*Avenella flexuosa*	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00000071	0.061254598	*Empetrum*	1.0000000	*Empetrum*	1.0000000
F_GH_00000002	0.056985307	Saliceae	1.0000000	Saliceae	1.0000000
F_GH_00000008	0.042779306	*Vaccinium*	1.0000000	Vaccinieae	1.0000000
F_GH_00000029	0.040685458	*Vaccinum ulignosum*	1.0000000	*Vaccinium*	1.0000000
F_GH_00000451	0.035570104	*Dicranum*	1.0000000	Dicranaceae	1.0000000
F_GH_00000110	0.024888587	*Dryopteris*	1.0000000	*Dryopteris*	1.0000000
F_GH_00000175	0.024263033	*Rumex*	1.0000000	Rumiceae	1.0000000
F_GH_00000974	0.023457761	Dicranaceae	1.0000000	Dicranidae	0.9444444
F_GH_00000618	0.016104741	*Dicranum*	1.0000000	*Dicranum*	1.0000000
F_GH_00000259	0.015280438	*Pinus*	1.0000000	*Pinus*	0.9777778
F_GH_00000019	0.013894326	Pooideae	1.0000000	Poeae	1.0000000
F_GH_00000262	0.013677626	Alopecurinae	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00000125	0.009579863	*Sagina*	1.0000000	Sagineae	1.0000000
F_GH_00000187	0.008594013	Hypnales	1.0000000	Hypnales	0.9545455
F_GH_00000137	0.006593659	*Bistorta vivipara*	1.0000000	Polygonoideae	1.0000000
F_GH_00000073	0.006558689	*Gymnocarpium dryopteris*	1.0000000	*Gymnocarpium dryopteris*	1.0000000
F_GH_00003467	0.006029129	*Equisetum*	1.0000000	*Equisetum*	1.0000000

Table B3.2. Overview of the top 20 most frequent MOTUs detected with the Euka02 primers.

MOTUs	Frequency	Scientific name (EMBL)	Rank	Best Identity
F_EU_00000002	0.310753564	leotiomyceta	No rank	0.9898990
F_EU_00000001	0.186588955	Fungi	Kingdom	0.9900000
F_EU_00000025	0.110369245	*Cordyceps memorabilis*	Species	0.9900000
F_EU_00000279	0.046502430	Agaricomycotina	Subphylum	0.9900000
F_EU_00000080	0.038141703	Agaricomycotina	Subphylum	0.9900000
F_EU_00000049	0.019856886	Dothideomycetes	Class	0.9898990
F_EU_00000098	0.018549859	*Preussia lignicola*	Species	1.0000000
F_EU_00000152	0.016967581	Venturiaceae	Family	1.0000000
F_EU_00000089	0.014422156	leotiomyceta	No rank	0.9898990

F_EU_00000369	0.011981321	Funariidae	Subclass	0.9905660
F_EU_00000483	0.010925582	Sordariomycetes	Class	0.9898990
F_EU_00000009	0.010808794	Embryophyta	No rank	0.9901961
F_EU_00000793	0.009760819	*Equisetum*	Genus	0.9803922
F_EU_00000912	0.009519886	*Strobiloscypha*	Genus	1.0000000
F_EU_00000017	0.009453775	*Lasiobulus*	Genus	1.0000000
F_EU_00000091	0.009314128	*Vaccinum macrocarpon*	Species	1.0000000
F_EU_00000281	0.008691004	Fungi	Kingdom	0.9898990
F_EU_00000087	0.006771057	Agaricomycotina	Subphylum	0.9702970
F_EU_00000020	0.006720561	*Psathyrella sp. D16*	Species	0.9900990
F_EU_00001846	0.006547246	*Rhizoctonia solani*	Species	1.0000000

Table B3.3. Overview of the top 20 most frequent MOTUs detected with the Fung01 primers.

MOTUs	Frequency	Scientific name (EMBL)	Rank	Best Identity
F_FU_00000010	0.300762436	*Thelebolus globosus*	Species	1.0000000
F_FU_00000003	0.158080022	*Glutinomyces*	Genus	0.9411765
F_FU_00000049	0.063483977	*Thelebolus*	Genus	1.0000000
F_FU_00000057	0.041588045	leotiomyceta	No rank	0.9116279
F_FU_00000001	0.030429674	*Thelebolus globosus*	Species	0.9948980
F_FU_00000200	0.029759841	*Thelebolus*	Genus	0.9897436
F_FU_00000203	0.026295701	Venturiaceae	Family	0.9641026
F_FU_00000233	0.016436326	*Preussia*	Genus	1.0000000
F_FU_00000343	0.014414004	*Kernia sp. 14PA07*	Species	0.9947917
F_FU_00000111	0.013951000	*Preussia sp. JJP-2009a*	Species	1.0000000
F_FU_00000598	0.013775578	*cf. Trechispora sp. 2 LF-2015*	Species	0.9221311
F_FU_00000316	0.013095907	Helotiales	Order	0.9595960
F_FU_00000026	0.012584439	*Schizothecium*	Genus	0.9400000
F_FU_00000002	0.012364520	*Cephalotrichum*	Genus	1.0000000
F_FU_00001861	0.012349689	*Schizothecium*	Genus	0.9359606
F_FU_00000308	0.011860248	Helotiales	Order	0.9497487
F_FU_00000314	0.009711708	Helotiales	Order	0.9497487

F_FU_00001167	0.008526530	*Hyaloscypha vitreola*	Species	0.9646465
F_FU_00002906	0.007876670	*Venturia hysterioides*	Species	0.9947917
F_FU_00001466	0.007818100	*Pseudeurotium*	Genus	1.0000000

Table B3.4. Overview of the top 20 most frequent MOTUs detected with the Bryo01 primers.

MOTUs	Frequency	Scientific name (EMBL)	Rank	Best Identity
F_BR_00000016	0.192429970	Hypnales	Order	0.9811321
F_BR_00000011	0.130481958	Hypnales	Order	0.9811321
F_BR_00000042	0.091090834	Polytrichaceae	Family	1.0000000
F_BR_00000014	0.078056025	*Dicranum*	Genus	1.0000000
F_BR_00000005	0.073054092	*Sphagnum*	Genus	1.0000000
F_BR_00000031	0.069510608	*Dicranum*	Genus	1.0000000
F_BR_00000017	0.057487184	Hypnales	Order	0.9811321
F_BR_00000006	0.050294250	*Pohlia nutans*	Species	0.9787234
F_BR_00000009	0.044429924	*Hamatocaulis*	Genus	0.9811321
F_BR_00000001	0.042436769	*Dicranum*	Genus	1.0000000
F_BR_00000010	0.024131162	Dicranaceae	Family	0.9795918
F_BR_00000092	0.022046092	Dicranaceae	Family	1.0000000
F_BR_00000095	0.013459867	Dicranum fuscescens var. Flexicaule	Varietas	1.0000000
F_BR_00000593	0.012766500	Bryaceae	Family	1.0000000
F_BR_00000084	0.011249042	Tayloria serrata var. Tenuis	Varietas	1.0000000
F_BR_00002083	0.009115151	*Marchantia polymorpha*	Species	1.0000000
F_BR_00000182	0.008834988	*Cryphaea tasmanica*	Species	0.9814815
F_BR_00000381	0.007553013	Jungermanniales	Order	0.9821429
F_BR_00000491	0.006578348	*Hylocomium splendens*	Species	1.0000000
F_BR_00000135	0.005658077	Dicranidae	Subclass	0.9795918

B4. Top 20 MOTUs detected with the G/H primer within the herbivores separately
Table B4.1 - B4.5. Overview of the top 20 most frequent MOTUs detected with the G/H primers within the herbivores separately (i.e. ”Ptarmigan”, ”Reindeer”, ”Moose”, ”Hare” and ”Rodent”. Abbreviations: C. = Chloroplast.

Table B4.1 – Overview of the top 20 most frequent MOTUs detected with the G/H primers in the ptarmigan diet.

MOTUs	Frequency	Scientific name (Ecochange)	Best identity (Ecochange)	Scientific name (EMBL)	Best identity (EMBL)
F_GH_00000001	0.729188295	*Betula*	1.0000000	Beluceae	1.0000000
F_GH_00000008	0.053007447	*Vaccinium*	1.0000000	Vaccinieae	1.0000000
F_GH_00000002	0.050645917	Saliceae	1.0000000	Saliceae	1.0000000
F_GH_00000125	0.026823617	*Sagina*	1.0000000	Sagineae	1.0000000
F_GH_00000047	0.026650518	Polypodiales	1.0000000	*Athyrium*	1.0000000
F_GH_00000029	0.025672381	*Vaccinium ulignosum*	1.0000000	*Vaccinium*	1.0000000
F_GH_00000259	0.017397846	*Pinus*	0.9487179	*Pinus*	0.9777778
F_GH_00000071	0.010852604	*Empetrum*	1.0000000	*Empetrum*	1.0000000
F_GH_00003793	0.008997546	*Micranthes stellaris*	1.0000000	*Micranthes stellaris*	1.0000000
F_GH_00000006	0.008081431	Polytrichaceae	0.9782609	Polytrichaceae	1.0000000
F_GH_00000137	0.006684005	*Bistorta vivipara*	1.0000000	Polygonoideae	1.0000000
F_GH_00000044	0.004772279	*Vaccinium*	1.0000000	*Vaccinium boninense*	1.0000000
F_GH_00000022	0.004579242	*Sciuro-hypnum*	1.0000000	Brachytheciaceae	0.9545455
F_GH_00001810	0.003432465	*Vaccinium vitis-idaea*	0.9791667	*Vaccinium vitis-idaea*	1.0000000
F_GH_00000020	0.002888685	*Avenella flexuosa*	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00002553	0.002318006	*Bryum*	1.0000000	Bryaceae	1.0000000
F_GH_00000041	0.002037254	*Cerastium fontanum*	1.0000000	*Cerastium*	1.0000000
F_GH_00001652	0.001983700	*Andromeda polifolia*	1.0000000	Vaccinioideae	1.0000000
F_GH_00002923	0.001807381	*Ranunculus*	1.0000000	*Ranunculus*	1.0000000
F_GH_00000340	0.001710537	*Vaccinium-vitis-idaea*	0.9800000	*Vaccinium-vitis-idaea*	0.9800000

Table B4.2 – Overview of the top 20 most frequent MOTUs detected with the G/H primers in the reindeer diet.

MOTUs	Frequency	Scientific name (Ecochange)	Best identity (Ecochange)	Scientific name (EMBL)	Best identity (EMBL)
F_GH_00000001	0.332935303	*Betula*	1.0000000	Betulaceae	1.0000000
F_GH_00003467	0.189459384	*Equisetum*	1.0000000	*Equisetum*	1.0000000
F_GH_00000029	0.131939952	*Vaccinium ulignosum*	1.0000000	*Vaccinium*	1.0000000
F_GH_00000562	0.105971762	Roisoideae	1.0000000	Rosaceae	0.9807692
F_GH_00000008	0.089256353	*Vaccinium*	1.0000000	Vaccinieae	1.0000000
F_GH_00000020	0.038802651	*Avenella flexuosa*	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00001712	0.020015668	*Cornus suecica*	1.0000000	*Cornus* suecica	1.0000000
F_GH_00000175	0.009927977	*Rumex*	1.0000000	Rumiceae	1.0000000
F_GH_00000259	0.009460971	*Pinus*	1.0000000	*Pinus*	0.9777778
F_GH_00000044	0.009458620	*Vaccinium*	0.9791667	*Vaccinium boninense*	1.0000000
F_GH_00000073	0.008694721	*Gymnocaprium dryopteris*	1.0000000	*Gymnocarpum dryopteris*	1.0000000
F_GH_00000312	0.007467183	*Juncus trifidus*	1.0000000	*Oreojuncus*	1.0000000
F_GH_00000071	0.007252170	*Empetrum*	1.0000000	*Empetrum*	1.0000000
F_GH_00001765	0.007041668	*Cornus suecica*	0.9800000	*Cornus suecica*	0.9800000
F_GH_00001551	0.006376614	*Arctous*	0.9795918	*Arctostaphylos uva-ursi*	0.9800000
F_GH_00096196	0.005245735	*Alchemilla*	1.0000000	*Alchemilla*	0.9795918
F_GH_00012178	0.003461841	Apiaceae	1.0000000	Apioideae	1.0000000
F_GH_00000019	0.002711832	Pooideae	1.0000000	Poeae	1.0000000
F_GH_00000471	0.002126155	Asteroideae	1.0000000	Asteroideae	1.0000000
F_GH_00000002	0.001817716	Saliceae	1.0000000	Saliceae	1.0000000

Table B4.3 – Overview of the top 20 most frequent MOTUs detected with the G/H primers in the moose diet.

MOTUs	Frequency	Scientific name (Ecochange)	Best identity (Ecochange)	Scientific name (EMBL)	Best identity (EMBL)
F_GH_00000047	0.346781801	Polypodiales	0.9487179	*Athyrium*	1.0000000
F_GH_00000002	0.244267119	Saliceae	1.0000000	Saliceae	1.0000000
F_GH_00000110	0.113922818	*Dryopteris*	1.0000000	*Dryopteris*	1.0000000
F_GH_00000001	0.085055334	*Betula*	1.0000000	Betulaceae	1.0000000
F_GH_00000187	0.049967551	Hypnales	1.0000000	Hypnales	0.9545455
F_GH_00000223	0.041419616	Maleae	1.0000000	Maleae	0.9803922

F_GH_00000259	0.029143213	*Pinus*	1.0000000	*Pinus*	0.9777778
F_GH_00000451	0.024413763	*Dicranum*	1.0000000	Dicranaceae	1.0000000
F_GH_00000821	0.019468634	Polypodiales	0.9230769	*Athyrium sinense*	1.0000000
F_GH_00000073	0.008964729	*Gymnocarpum dryopteris*	1.0000000	*Gymnocarpum dryopteris*	1.0000000
F_GH_00000020	0.007594707	*Avenella flexuosa*	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00000022	0.003781478	*Sciuro-hypnum*	1.0000000	Brachytheciaceae	0.9545455
F_GH_00004013	0.003103449	*Juniperus*	1.0000000	Cupressaceae	0.9750000
F_GH_00000553	0.002715825	*Phegopteris connectilis*	1.0000000	Polypodiales	0.9487179
F_GH_00000008	0.002381414	*Vaccinium*	1.0000000	Vaccinieae	1.0000000
F_GH_00002144	0.001836962	*Sphagnum russowii*	1.0000000	*Sphapgnum*	1.0000000
F_GH_00000279	0.001579980	*Eriophorum*	1.0000000	*Eriophorum*	1.0000000
F_GH_00000019	0.001454192	Pooideae	1.0000000	Poeae	1.0000000
F_GH_00003167	0.001213563	*Festuca*	1.0000000	Pooideae	1.0000000
F_GH_00078869	0.000997696	*Tetraplodon pallidus*	1.0000000	*Tetraplodon pallidus*	1.0000000

Table B4.4 – Overview of the top 20 most frequent MOTUs detected with the G/H primers in the hare diet.

MOTUs	Frequency	Scientific name (Ecochange)	Best identity (Ecochange)	Scientific name (EMBL)	Best identity (EMBL)
F_GH_00000001	0.170040309	*Betula*	1.0000000	Betulaceae	1.0000000
F_GH_00000020	0.165649000	*Avenella flexuosa*	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00000029	0.144334628	*Vaccinium ulignosum*	1.0000000	*Vaccinium*	1.0000000
F_GH_00000008	0.200636213	*Vaccinum*	1.0000000	Vaccinieae	1.0000000
F_GH_00000019	0.092299737	Pooideae	1.0000000	Poeae	1.0000000
F_GH_00000262	0.064751282	Alopecurinae	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00000002	0.064399630	Saliceae	1.0000000	Saliceae	1.0000000
F_GH_00000047	0.035293849	Polypodiales	0.9487179	*Athyrium*	1.0000000
F_GH_00001167	0.018944290	Asteraceae	1.0000000	Asterales	0.9803922
F_GH_00000071	0.017153507	*Empetrum*	1.0000000	*Empetrum*	1.0000000
F_GH_00000525	0.013139898	Pooideae	1.0000000	Poaceae	1.0000000
F_GH_00001622	0.011739923	Agrostidinae	1.0000000	Poeae C. Group 1 (Aveneae type)	1.0000000
F_GH_00001810	0.010655891	*Vaccinium vitis-idea*	1.0000000	*Vaccinium* vitis-idea	1.0000000
F_GH_00000471	0.009632606	Asteroideae	1.0000000	Asteroideae	1.0000000

F_GH_00000044	0.008902445	*Vaccinium*	0.9791667	*Vaccinium boninense*	1.0000000
F_GH_00000259	0.007582515	*Pinus*	1.0000000	*Pinus*	0.9777778
F_GH_00005992	0.006998828	*Carex*	1.0000000	*Carex*	1.0000000
F_GH_00003167	0.006533776	*Festuca*	1.0000000	Pooideae	1.0000000
F_GH_00000284	0.004972340	Poeae	1.0000000	Poeae	1.0000000
F_GH_00000873	0.004750690	Poeae	1.0000000	Poeae	1.0000000

Table B4.5 – Overview of the top 20 most frequent MOTUs detected with the G/H primers in the rodent diet.

MOTUs	Frequency	Scientific name (Ecochange)	Best identity (Ecochange)	Scientific name (EMBL)	Best identity (EMBL)
F_GH_00000001	0.155482300	*Betula*	1.0000000	Betulaceae	1.0000000
F_GH_00000071	0.147794709	*Empetrum*	1.0000000	*Empetrum*	1.0000000
F_GH_00000020	0.121222962	*Avenella flexuosa*	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00000047	0.088593437	Polypodiales	0.9487179	*Athyrium*	1.0000000
F_GH_00000451	0.088233043	*Dicranum*	1.0000000	Dicranacrae	1.0000000
F_GH_00000175	0.063916266	*Rumex*	1.0000000	Rumiceae	1.0000000
F_GH_00000974	0.063083855	Dicraneceae	1.0000000	Dicranidae	0.9444444
F_GH_00000618	0.043077104	*Dicranum*	1.0000000	*Dicranum*	1.0000000
F_GH_00000110	0.031279002	*Dryopteris*	1.0000000	*Dryopteris*	1.0000000
F_GH_00000029	0.024555165	*Vaccinium ulignosum*	1.0000000	*Vaccinium*	1.0000000
F_GH_00000008	0.021772127	*Vaccinium*	1.0000000	Vaccinieae	1.0000000
F_GH_00000262	0.014408320	Alopecurinae	1.0000000	Poeae C. Group 2 (Poeae type)	1.0000000
F_GH_00000073	0.013637164	*Gymnocarpum dryopteris*	1.0000000	*Gymnocarpum dryopteris*	1.0000000
F_GH_00000259	0.012091317	*Pinus*	1.0000000	*Pinus*	0.9777778
F_GH_00000137	0.011323482	*Bistorta vivipara*	1.0000000	Polygonoideae	1.0000000
F_GH_00001810	0.007568497	*Vaccinium vitis-ideae*	1.0000000	*Vaccinium vitis-ideae*	1.0000000
F_GH_00000002	0.007132866	Saliceae	1.0000000	Saliceae	1.0000000
F_GH_00000187	0.005727997	Hypnales	1.0000000	Hypnales	0.9545455
F_GH_00000019	0.004786049	Pooideae	1.0000000	Poeae	1.0000000
F_GH_00000312	0.004458361	*Juncus trifidus*	1.0000000	*Oreojuncus*	1.0000000