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DNA metabarcoding of fecal samples reveals seasonal and inter-annual variability in willow ptarmigan diet

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Preface

This thesis represents the end of my master's degree in biosciences with a specialization in terrestrial ecology and nature management. It has been five great years at Nord university filled with knowledge, experiences, and friendship. The thesis is a part of the project "Willow Ptarmigan ecology in a changing climate" conducted by Norwegian Institute for Nature Research (NINA) and Nord University and is financed by Norwegian Environment Agency.

I would like to thank Nord University and NINA for the opportunity to be a part of their project with all that has been involved from experiences in the field and in the laboratory, to working with the master's thesis. I am humbled by all the knowledge around me, and grateful for how willing people have been to share their knowledge and to contribute with help when needed.

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Abstract

Herbivore species living in alpine environments have limited access to nutritious food during large parts of the year due to snow cover and a short plant growing season. In alpine ecosystems global warming is expected to result in earlier onset of spring and longer growing season. Access to nutrient-rich plant materials in late winter and early spring is essential for survival and reproduction of resident herbivore birds. Understanding the spatio-temporal variation in the diet of alpine herbivores is important to understand how a changing climate will affect these species in the future.

In this study, I carried out DNA metabarcoding of fecal pellets (n=99) from willow ptarmigan (*Lagopus lagopus*) collected in central Norway from March to June over three consecutive years (2019–2021). Analyses of fecal pellets sampled during the transition from winter to late spring provided a unique insight into the diet and its seasonal and inter-annual variation. By using remote sensing tools to create seasonal variables for the abiotic factors snow cover and vegetation phenology, it was assessed how these factors can affect variations in the diet. Because male and female willow ptarmigan have different energetic requirement and habitat selection in the spring, it was also investigated whether the diet composition differs between the two sexes.

A total of 18 important diet components were documented in this study. The three most frequently occurring genera in the diet were Betula, Vaccinium and Empetrum. Both diet composition and richness varied seasonally and inter-annually. Seasonally, a shift from a narrow winter diet dominated by trees and dwarf shrubs to a broader spring diet with increasing elements of more nutritious field vegetation was evident. The seasonal progression differed inter-annually. The variation in diet across time were to a larger extent explained by day of year than by snow cover and vegetation phenology. Females had a more diverse diet than males, but there were no differences in diet composition of females and males across all fecal samples. This study demonstrates the benefits of using metabarcoding of fecal samples in dietary studies. Sampling of fecal pellets over temporal and spatial scales provides the opportunity to assess what factors affect diet composition of species in alpine ecosystems, and to study temporal and spatial shifts in diet in the context of a changing climate.

Key words: alpine bird, Lagopus lagopus, willow ptarmigan, metabarcoding, dietary study, environmental DNA, herbivory, phenology, NDVI, NDSI.

Sammendrag

På grunn av snødekke og kort vekstsesong har herbivore arter i alpine omgivelser store deler av året begrenset tilgang på næringsrik mat. I alpine økosystemer forventes global oppvarming å føre til en tidligere vår og en lengre vekstsesong. For herbivore standfugler er tilgang til næringsrikt plantemateriale om våren essensielt for overlevelse og reproduksjon. For å forstå hvordan et klima i endring vil påvirke herbivore arter i alpine områder er det viktig å forstå hvordan artenes diett varierer over tid og rom.

I denne studien gjennomførte jeg DNA-strekkoding av ekskrementer (n=99) fra lirype (*Lagopus lagopus*), samlet i Midt-Norge fra mars til juni over tre påfølgende år (2019–2021). Analyser av ekskrementer fra overgangen mellom vinter til sen vår ga et unikt innblikk i lirypas diett og hvordan dietten varierer mellom sesong og år. Det ble ved hjelp av fjernanalyse konstruert sesongvariabler for de abiotiske faktorene snødekke og vegetasjonsfenologi, for å undersøke hvordan disse faktorene påvirker variasjon i dietten. Fordi høne og stegg hos lirype har ulikt energibehov og habitatvalg om våren, ble det også undersøkt om kjønnene har ulik sammensetning av diett.

Totalt 18 viktige diettkomponenter ble dokumentert i dette studiet. De tre hyppigst forekommende slektene i dietten var Betula, Vaccinium og Empetrum. Både sammensetning og mangfold i dietten varierte sesongmessig og mellom år. Sesongmessig var det et tydelig skifte fra en smal vinterdiett dominert av trær og dvergbusker til en bredere vårdiett med økende innslag av mer næringsrik felt-vegetasjon. Den sesongmessige progresjonen i kosten var ulik mellom år. Den tidsmessige variasjonen i dietten ble i større grad forklart av dag på året enn av snødekke og plantefenologi. Lirype-høna hadde en mer variert diett enn steggen, men det var ingen forskjeller i diettsammensetning hos høne og stegg på tvers av alle ekskrementprøver. Dette studiet demonstrerer fordelene ved å bruke DNA-strekkoding av ekskrementprøver i diettstudier. Ekskrementprøver som er samlet over tidsmessige og romlige skalaer gir muligheter for å vurdere hvilke faktorer som påvirker diettsammensetningen til arter i alpine økosystemer, og også å studere tidsmessige og romlige endringer i dietten i sammenheng med et endret klima.

Stikkord: alpin fugl, Lagopus lagopus, lirype, DNA-strekkoding, diettstudie, miljø-DNA, herbivore, fenologi, NDVI, NDSI.

Index

1. Introduction

For species living in seasonal environments, it is fundamental to time periods of somatic growth, reproduction, and energy storage with the resources available during the productive season (Varpe, 2017). This particularly applies to herbivore species in alpine ecosystems, where snow cover and short plant growing season make access to nutritious food a limited factor (Rixen et al., 2022). Changes in plant phenology caused by climate warming are well documented (IPCC, 2021), and the most pronounced changes are seen in alpine areas where timing and duration of snow cover affect plant growth and influence vegetation composition (Burrows et al., 2011). In the alpine ecosystems in Fennoscandia, global warming is expected to result in an earlier onset of the spring and a longer growing season (Forsgren et al., 2015). This change in plant phenology could affect life history traits of herbivore species living in such habitats (Pettorelli et al., 2005). For herbivorous resident bird species living in seasonal environments, increased temperature and earlier snow melt could lead to an earlier access to green nutrient-rich plant material in the spring and the opportunity to benefit from a longer growing season (Ernakovich et al., 2014). Reconstruction of the diet of alpine species, and knowledge about spatiotemporal factors affecting their diet, is important to understand species interactions and ecosystem functions under novel and chancing conditions resulting from the ongoing climate change (Clare, 2014).

Willow ptarmigan (*Lagopus lagopus*) is an herbivorous bird with a circumpolar arctic distribution. In mountainous areas in Fennoscandia willow ptarmigan is typically inhabiting the subalpine to alpine bioclimatic zone, where their habitats are located in the mountain birch forest zone and upwards (Kvasnes et al., 2018). It is a keystone species in the alpine ecosystem, and an important prey for mountain predators (Valkama et al., 2005; Bowler et al., 2020). In addition, the willow ptarmigan is a highly valued small game species (Aanes et al., 2002). Globally, the willow ptarmigan is considered as least concern (LC) in the International Red List of Species (BirdLife International, 2021), but locally there are concerns related to declining populations and disrupted cyclicity (Fuglei et al., 2020). In Norway, it has been a clear decline in the population of willow ptarmigan over a long time-period, but there has been a stabilization of the population in recent years (Stokke et al., 2021; Fuglei et al., 2020).

Willow ptarmigan is mainly a resident bird, and the majority of the individuals stay in the same area throughout the year, but in some mountain areas seasonal migratory movements between nesting areas and winter areas can occur (Arnekleiv et al., 2022). As a resident species, willow

ptarmigan life history traits are adapted to living in a seasonal ecosystem, and their diet throughout the year reflects this. During the winter season their habitats are to a large extend covered by snow, and since willow ptarmigan forage mostly on the ground, their foraging behavior is strongly dependent on snow level (Hakkarainen et al., 2007). To a large extent they utilize the plants that are available above the snow cover as food resources, and their digestive system enables them to survive the winter on a very lean plant diet (Pedersen & Karlsen, 2007). Throughout the winter, previous studies of willow ptarmigan have suggested that they feed almost exclusively on shoots and catkins of mountain birch (*Betula pubescens subsp. Czerepanovii*) and shoots and flower buds of willows (*Salix spp*.) and bilberry (*Vaccinium myrtillus*) stems (Pedersen & Karlsen, 2007; Watson & Moss, 2008).

Plant phenology and vegetation composition in the ptarmigan habitats are tightly linked to the distribution and timing of snow (Rixen et al., 2022), and throughout spring snow cover influence which plant species are available as food (Garcia-Gonzales et al., 2016). Because of higher nutritious value, willow ptarmigan switch to feeding on field layer vegetation when the first snowless spots appear (Pulliainen & Tunkkari, 1991). This shift leads to a variation in diet richness and composition between seasons (Brittas, 1988). Nutritious field layer vegetation represents the main food resource during the egg-laying period, and the diet composition is a significant ecological factor that affect the breeding success and survival of ptarmigans (Moss & Watson, 1984; Brittas, 1988). Previous studies have shown that the females condition affects their later breeding success. Females loses weight during the incubation period, and those that have early access to green plant material in the spring will be in better condition during the breeding period and thereby have more energy reserves for egg production and incubation (Moss et al., 1975; Brittas, 1988). Gardarsson & Moss (1970) also found that egg quality is influenced by the diet of laying hens, and especially the amount of nutritious newly grown plants that are available.

Because snow cover and plant phenology will have an inter-annual spatial and temporal variation, there will also be an inter-annual variation in the types of plant food available for willow ptarmigan at different times of the year. In years with early snowmelt, they can benefit from a longer period of high-quality food resources while in years with late snowmelt a poorer diet can lead to poorer condition of the birds (Garcia-Gonzales et al., 2016b). Climate change is expected to affect timing of snow and vegetation phenology, and potentially also affect the ptarmigan temporal variation in diet, which in turn could affect their reproduction. A warmer climate that provides earlier access to fresh nutritious plants can thus have a positive effect for

ptarmigans, at least on a short-term basis (Bowler et al., 2021). Even though earlier snowmelt and greening can be advantageous for willow ptarmigan in terms of food supply, climate change can also pose several challenges. For example, more frequent occurrences of rain on snow events during the winter can lead to an ice layer over the vegetation that makes most of the food resources inaccessible to the birds (Eidesen et al., 2020).

Males and females have different energy requirements because of their different behavior during the breeding season. Males start their territorial calling and habitat defense from March (Pedersen et al., 1983). During the spring period males therefore eat less and lose weight (Savory, 1983). At the same time, it is important for the females to build up their body reserves to prepare them for egg-laying and hatching, and this difference between the sexes is known to lead to a sex-specific foraging behavior and habitat selectivity (Gardarsson & Moss, 1970; Gruys, 1993; Elson et al., 2007). Despite these behavioral differences, several studies have not been able to demonstrate any sex differences in the diet composition of ptarmigan during the winter and spring seasons (Brittas 1984; Pulliainen & Trunkkari, 1991; Garcia-Gonzales et al., 2016).

Although spring diet is known to be an important factor affecting condition and breeding success in willow ptarmigan, information about how the willow ptarmigan utilizes the plant diet in different parts of the spring and which factors that affect their diet at this time of year is scarce. Recent developments in DNA metabarcoding have opened up new possibilities for detailed examination of the temporal variation in diet. (Sousa et al., 2019). Dietary analyzes using DNA metabarcoding of fecal samples have previously been applied in studies of svalbard rock ptarmigan (*Lagopus muta hyperborea*) (Eidesen et al., 2020) and japanese rock ptarmigan (*Lagopus muta japonica*) (Fujii et al., 2019). However, to my knowledge, DNA metabarcoding has not been used in dietary analyzes of willow ptarmigan.

The aim of the present study is to investigate the diet composition and richness of a willow ptarmigan population in Central Norway, during the transition period from winter to late spring, based on DNA metabarcoding of fecal pellets. I predict that willow ptarmigan has a narrow winter diet where mountain birch will be the dominant diet component, and that the species shifts to a broader spring diet with increasing elements of nutritious field vegetation as the spring progresses. Further, I predict that this increase in nutritious field vegetation and shift in diet components can be explained by snow cover and vegetation phenology in the study area. I expect that since these abiotic factors will occur at different times each year, there will be an

inter-annual variation in the diet composition. In addition, because of different energetic requirement and habitat selection between the sexes in this time of the year, I predict that the diet composition of the males and females will differ. This study will provide insight into how future climate projections in alpine environments can affect an already vulnerable species and provide knowledge for effective management and conservation strategies.

2. Materials and methods

2.1 Study site

Data were collected at Lifjellet (64°27 N, 13°14 E) in Lierne municipality, central Norway (Figure 1), during the transition from winter to late spring over three consecutive years (2019–2021). Lifjellet is situated in the subalpine to alpine bioclimatic zone (Moen, 1999). The lower part of the study area is dominated by sparse forest with Norway spruce (*Picea abies)*, and Scots pine (*Pinus sylvestris)*, interspersed with mountain birch and *Salix* spp. Middle parts of the area consist of more open mountain vegetation with dwarf birch (*Betula nana)* and scattered patches of a few other tree species. Large parts of the middle areas consist of bogs covered by grasses and sedges, interspersed with drier areas with ericaceous dwarf shrubs. At the highest elevations, the landscape alternates between exposed ridges, leesides with dwarf shrubs, snowbeds and shallow bogs.

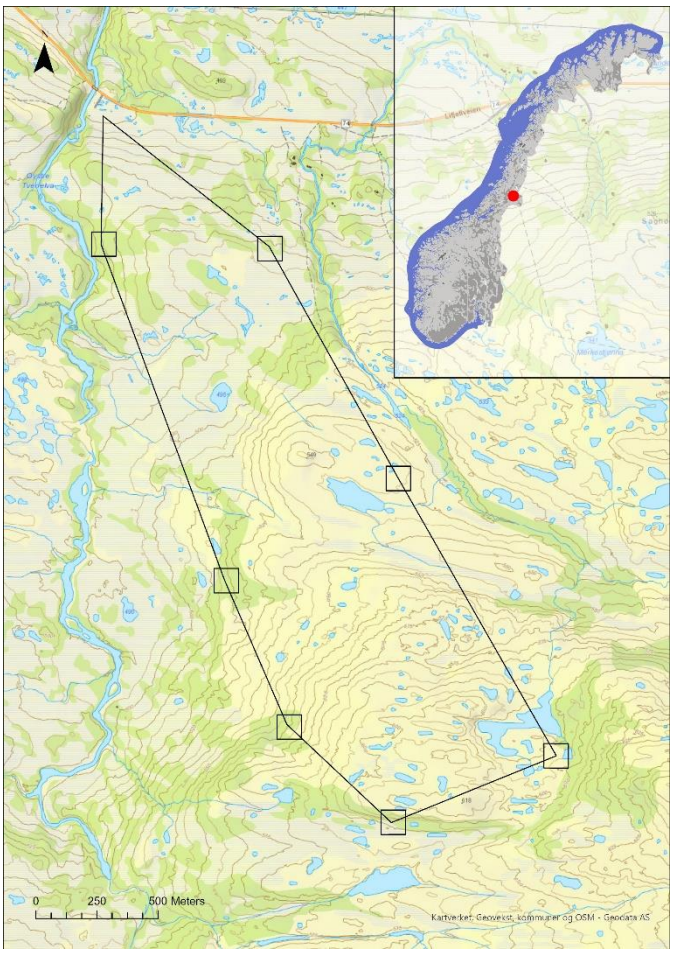

Figure 1. The location of the study area in central Norway. Shown is also the transect and the seven squares used for collection of fecal pellets

Annual average precipitation in the area is approx. 675 mm per year (Nilsen et al., 2020). Temperatures span from an annual average of -10 ˚C in January to 12 ˚C in July. In winter, snow dept is 1-3 meters, and snow cover typically persist from early October to late May (Israelsen et al., 2021). Main predators on willow ptarmigan in the study area are gyrfalcon (*Falco rusticolus),* golden eagle (*Aquila chrysaetos*) and red fox (*Vulpes vulpes).* Arctic fox (*Vulpes lagopus),* lynx (*Lynx lynx),* and wolverine (*Gulo gulo)* are also present in the study area, but due to low densities they do not represent a major predator risk (Israelsen et al., 2020).

2.2 Fecal collection

Fresh fecal pellets from willow ptarmigan were collected once a week from March to June, by two field workers in seven predefined sampling plots with an area of 100 m x 100 m across an altitude gradient. The sampling plots were connected by a predefined transect with a total distance of seven km (Figure 1). Fecal pellets were collected within each of the sampling plots, and we systematically searched for fecal pellets by walking back and forth in parallel lines from one end of the square to the other. In each square an upper limit of 15 minutes was set for searching and sampling of pellets. Along the transect, fecal pellets were collected when they were found at random. A handheld GPS was used to follow the transect and to identify the boundaries of the squares, and track logs from each field trip and geographical coordinates for samples collected along the transect were stored. Because DNA may degrade as the fecal pellets age, and because we wanted to be able to determine the date the pellet was dropped as accurately as possible, a main inclusion criterion was to collect pellets that were assumed to be no older than 0-3 days. In an attempt to meet this criterion, we used three different decision keys: First, pellets were always collected from willow ptarmigans observed along the transect and within the squares. Second, pellets found on or close to fresh tracks were also considered as fresh. In addition, we used the appearance of the pellets to evaluate the age, based on pellet color and texture. An assessment of the accuracy of the pellet date was always noted in the field form (Appendix A). The willow ptarmigan inhabits areas that overlap with the sympatric rock ptarmigan *(Lagopus muta*), and it is difficult to distinguish between pellets of the two species based on visual appearance. Therefore, species identity was determined based on DNA microsatellite analyses (see chapter 2.3). After collection, pellets were put into sterile airtight tubes containing silica gel (granulate size 1-3 mm from Chameleon in 2019 and 2020, granulate size 2–5 mm from Real Marine in 2021) for desiccation, and only one pellet was stored in each tube. To minimize the chance of collecting multiple samples from the same individual on the same day only fecal pellets that were found at least five meters apart were collected. A

maximum of five fecal pellets were collected from each sampling plot at each visit since this was assumed to provide representative samples for each square. After collection, the sample tubes were stored under dry and dark conditions at room temperature prior to DNA extraction.

During this study we collected a total of 399 fecal samples from ptarmigans (140 in 2019, 187 in 2020 and 72 in 2021). The collected samples were organized into seven equal time periods (blocks), and from these blocks, 141 samples were selected for DNA extraction. We selected pellets that were assumed to be as fresh as possible, and we subsampled samples across years and periods to achieve a balanced design (i.e., equal number of samples from each of the seven blocks and from each year). For interpretation of results and for use in further analyzes, the samples were also organized into three different time periods, winter (block 1-2), early spring (block 3-4) and late spring (block 5-7).

2.3 DNA extraction and library preparations

Extraction of DNA and library preparations were carried out at the Norwegian Institute for Nature Research (NINA). DNA was extracted from the fecal pellets using the MP Biomedicals™ FastDNA™ SPIN Kit for Soil (MP Biomedicals, Germany) following the manufacturers protocol. A panel of nine variable microsatellite markers was used to determine species and individual identity. An additional marker was included for sex determination. The panel contained markers that amplify relatively short fragments (< 300 base-pairs, bp), as DNA might be degraded in fecal pellets (Taberlet et al., 1999). The microsatellite loci were amplified in two multiplex sets by polymerase chain reaction (PCR) and using fluorescently labeled forward primers. The alleles were separated by capillary electrophoresis on an ABI3500xl Genetic Analyzer, and the sizes determined with the software GeneMapper. Each fecal sample was genotyped three times and from these replicates a consensus genotype was constructed by applying the following criteria: loci with a heterozygote result had to show this in two independent PCRs while loci with a homozygote result had to show this in three independent PCRs. Consensus genotypes containing at least eight loci were included for individual identification and species determination. Allelematch (Galpern et al., 2012) was used to identify unique genotypes. The nine microsatellite markers amplify DNA from both willow ptarmigan and rock ptarmigan, but the allele frequencies are different for the two species. To determine whether the samples were from willow ptarmigan or rock ptarmigan, the DNA profiles of the fecal samples were compared with DNA profiles from a reference material from both ptarmigan species using the population assignment function in GenAlEx v6.501 (Peakall & Smouse, 2012).

To characterize the diet of the willow ptarmigan, an ITS2 universal primer set for plants was used. The ITS region of rDNA was amplified using the ITS2-S2F (5'-ATGCGATACTTGGTGTGAAT-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. PCR was conducted in 25 µL volumes containing: 1X KAPA HiFi HotStart Ready Mix, 0.5 µM of each primer, and 2.5 µL of 10 ng/µL template DNA. The thermal cycling profile was 95 °C for 3 min, 95 °C for 30 s, 35 cycles of 56 °C for 30 s, 72 °C for 30 s, and a final extension of 10 min at 72 °C. IDT for Illumina DNA/RNA UD indices were added to the 5' and 3' ends of the amplicons according to the manufacturer's instructions. Amplicons were diluted to 7ng/µL, and magnetic beads were used to remove fragments under 200 bp and over 600 bp. Amplicons were pooled in equimolar amounts and sequenced on an Illumina NovaSeq 6000 platform at the Norwegian Sequencing Centre.

Demultiplexing of the samples and adapter removal was conducted on the Illumina MiSeq platform. Cuadapt v.1.18 (UNIX, Martin, 2011) was used to remove the 5' primer and the 3' primer and any additional index and adapter bases, requiring a minimum length match of 17 bp with 0.15 expected errors. Quality filtering, error correction, merging, mapping and chimera removal were all conducted using the DADA2 v.1.22 package for R (Callahan, 2016). Reads were quality-filtered to remove all sequences with ambiguous bases, with >2 expected errors in both the forward and reverse direction, and length <50 bp after truncation at the first instance of a base with a quality score <10. Error rates were estimated for forward and reverse sequences, forward and reverse reads were merged with a minimum overlap of 30 bp, and amplicon sequence variants (ASVs) were generated for each sample. Sequence variants that were flagged as chimeric in more than 90 % of the samples were removed from the dataset. Taxonomy was assigned using the Sintax algorithm (Edgar, 2016) and a custom database of publicly available ITS sequences for Norwegian plant species with a requirement of >80 % confidence for successful assignment at a given taxonomic level. Megablast comparisons to GenBank were used to identify and remove non-target ASVs (non-Streptophyta).

From the 141 fecal pellets sampled for DNA analysis, DNA was successfully extracted in 121 samples and resulted in DNA quality sufficient for species, sex, and individual determination. Based on the microsatellite data, 22 of these 121 fecal pellets were identified as pellets from rock ptarmigan. Final dataset thus consisted of 99 willow ptarmigan fecal samples, 50 from males and 49 from females (Table 1). From these samples, 86 amplicon sequence variants (ASVs) were kept after taxonomic assignment (Appendix B). Of the 86 ASVs, 5 (5.8 %) were assigned to family level, 21 (24.4 %) to genus level and 60 (69.8 %) to species level. The ASVs

were further grouped into six different functional groups; forbs, trees, graminoids, dwarf shrubs and bryophytes. Some of the detected species were obviously unlikely for the study area, for instance cauliflower and cucumber. To identify and exclude species that are unlikely to occur in the study area, I used the Species Map Service for Norway (artskart.artsdatabanken.no) and consulted a professional botanist from Nord university (Håkon Holien). Based on this, 27 of 86 ASVs were identified as unlikely for the study area. To exclude false positives in the result, I removed all species that had the same or less total sequence amount than the false positive species with the highest total amount (0.014 %). All other species that were removed in this operation consisted of a very small proportion of the sequences and thus were considered not to be important dietary components.

Table 1. Final dataset of 99 fecal pellets from willow ptarmigan divided into years and blocks. Block seven was not sampled in 2019.

Year	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7
	26.03-04.04	09.04-17.04	23.04-03.05	07.05-16.05	21.05-30.05	04.06-14.06	18.06-26.06
2019	6	6	5	7	3	1	0
2020	4	7	7	2	5	8	6
2021	3	7	7	7	4	3	1

2.4 Data preparation

2.4.1 Construction of seasonal variables

To examine how the seasonal variables affect the diet, three seasonal covariates were established for the further analyses: Julian date, Normalized difference vegetation index (NDVI) and Normalized difference snow index (NDSI). NDVI was used to calculate the expansion of green biomass in the study area. NDVI is a vegetation index that provides ratios based on radiometric values for reflected sunlight, reflected by the vegetation and captured by the sensor of the satellite, in different spectral registration bands (Pettorelli et al., 2005). The use of this index makes it possible to show relative density and growth activity for green vegetation across time and space (Pettorelli et al., 2005). NDVI makes use of band near infrared (NIR) and red and is calculated by the equation; NDVI = $\frac{(NIR - Red)}{(NIR + Red)}$ Furthermore, to explore the snow cover in the study area, the Normalized Difference Snow Index (NDSI) was used, NDSI is a measure of the relative magnitude of the reflectance difference between the visible

(green) band and shortwave infrared (SWIR) band, and is calculated by the equation; NDSI = $\frac{(Green - SWIR)}{(Green + SWIR)}$ (Salomonsen & Appel, 2004).

NDVI and NDSI were constructed from Sentinel 2 satellite imagery using the Google Earth Engine platform. The geometry boundary for image collection was defined by a polygon that represented the study area with a 200 meter buffer zone around. The satellite imagery collection was further filtered on date and year for the collection periods, and all available images from the relevant periods were selected. To filter out clouds, water and shadows from the images, an algorithm described by Hollstein et al. (2016) was used. NDVI and NDSI values were extracted from 10 000 random points in the study area from the imagery collection. Further processing of the values was carried out using R version 4.1.2 (R Development Team 2021). The R-package *xts* (Ryan & Ulrich, 2020) was used to create extensible time-series object which constructed weekly averages for NDVI and NDSI for each week of sample collection. Further, the function gam in R-package *mgcv* (Wood, 2011) was used to fit generalized additive models (GAM) for NDVI and NDSI values for each year. From these models I retrieved predicted values for NDVI and NDSI for each sampling date.

Snow cover and the arrival of spring varied between the three years of data collection (Figure 2 and 3). In 2019 snow cover was sparse and this led to an early snowmelt and greening in the study area, in 2020 large parts of the study area were covered by snow until the middle of June, while 2021 represented an average between the two previous years.

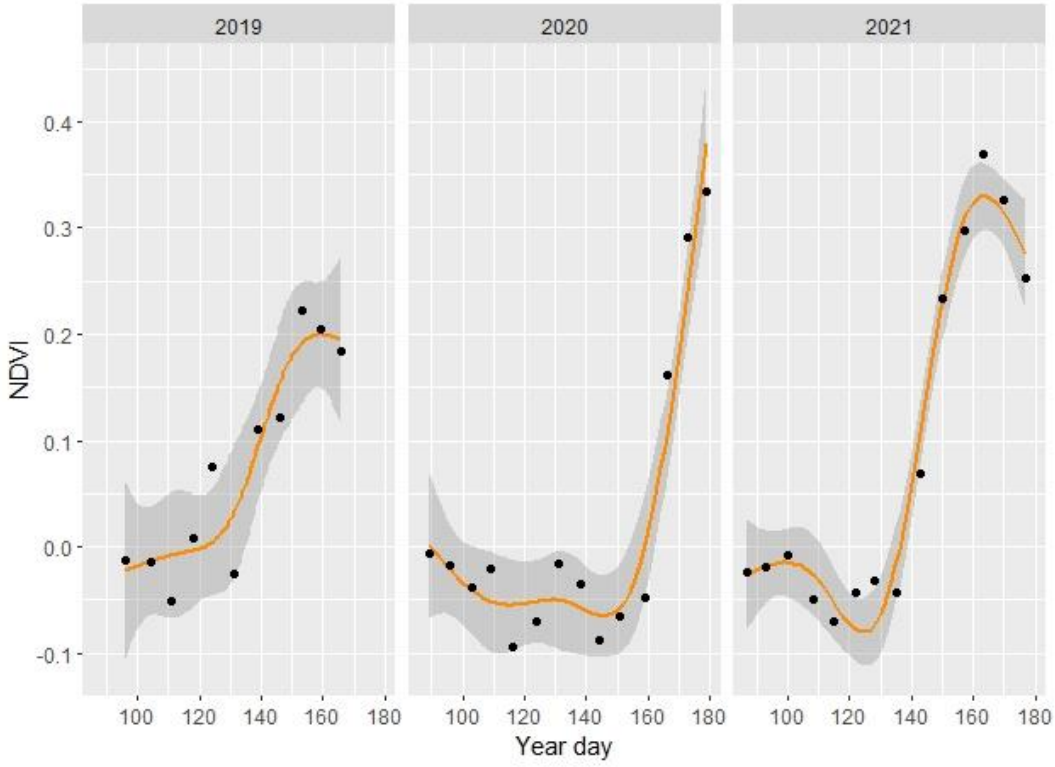

Figure 2. Estimated relationship (solid line) between predicted NDVI-values and day of the year. The shaded ribbons represent 95% confidence interval. The points represent sampling day.

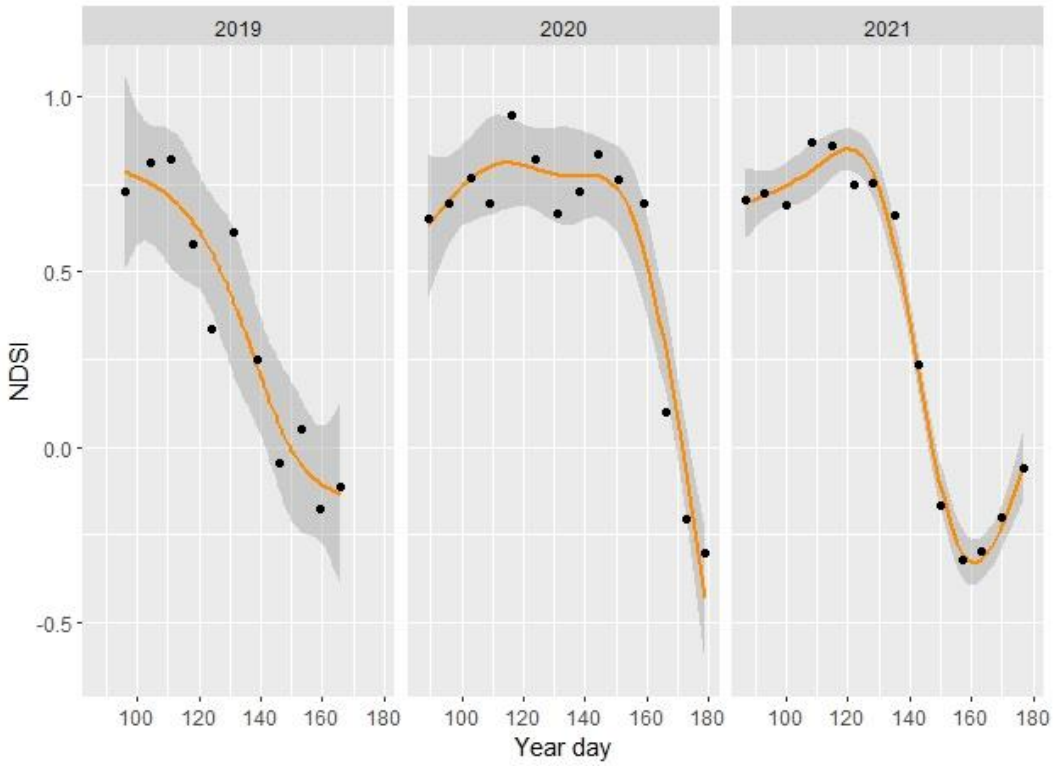

Figure 3. Estimated relationship (solid line) between predicted NDVI-values and day of the year. The shaded ribbons represent 95% confidence interval. The points represent sampling day.

2.4.2 Quantifying the ptarmigan diet

Mainly two indicators have been used to quantify dietary data in fecal metabarcoding studies: Frequency of occurrence (FO) and relative read abundance (RRA) (Ando et al., 2020). To calculate diet richness, I used the frequency of occurrence metrics (FO), which represent presence/absence of each taxa in each fecal pellet (Ando et al., 2020). To calculate dietary composition, I used the relative read abundance (RRA), defined as the proportion of identified sequence reads for each plant taxa in a sample (Ando et al., 2020). Both indicators have some known potential biases: FO can overestimate the importance of plant taxa eaten only in small amounts, while RRA does not necessarily reflect the exact proportion of consumed plant taxa (Deagle et al., 2018). To reduce biases by reporting only one, I used both dietary metrics.

2.5 Statistical analyses

As an initial test of how dietary composition of willow ptarmigan in our study area varied across time, I first focused on the FO and RRA of the three most frequent genera across all samples in the willow ptarmigan diet (Betula, Vaccinium and Empetrum, see Table 2). For each taxa and for both FO and RRA, I constructed and compared ten different candidate generalized linear models (glm). The ten candidate models included three models with the three different season variables as single effects (Julian date, NDVI and NDSI, respectively), three models with the three different season variables and year as an additive effect, three models with the season variables and sampling year as both an additive and interaction effect, and a null (i.e. intercept only) model. The only exception was for Vaccinium FO where one of the candidate models (Vaccinium FO ~ jDate + Year + jDate×Year) was excluded due to Hauck-Donner effect. For the FO-data I used generalized linear models with binary response (1 = presence in sample, 0 = absence in sample) and logit link function, assuming binomial error distribution. For the RRA-data I used general linear models with continuous response and identity link function, assuming Gaussian error distribution. Because the RRA is a proportion between 0 and 1, I logit-transformed the response variable prior to analyses (Warton & Hui, 2011). For model selection I used the Akaike Information Criterion corrected for small sample sizes (AICc) (Akaike, 1974). I considered the less complex model within a ΔAICc range of 0-2 as the most parsimonious model and considered models with a ΔAICc value > 2 as inconclusive (Burnham & Anderson, 2004). For the continuous models, I evaluated model fit by visual interpretation of residual plot and Shapiro-Wilk test >9.

As a measure of diet richness, I used FO-data to calculate number of species, number of families and number of functional groups per fecal sample. To examine the predictors of the diversity

of species per sample I used generalized linear models with a count response and log link function. I constructed and compared 19 different candidate models. The 19 candidate models included three models with the three different season variables as single effects (Julian date, NDVI and NDSI, respectively), three models with the three different season variables and year as an additive effect, three models with the season variables and sampling year as both an additive and interaction effect, three models with the different season variables and sex as an additive effect, three models with the season variables and with sex and sampling year as additive effects, three models with the season variables and with sex as an additive effect and sampling year as both an additive and interaction effect, and a null (i.e. intercept only) model. Model selection was done as described earlier, but in this case also with sex as an additive effect. Because of underdispersion in the species count data I used a Conway–Maxwell–Poisson distribution that includes an additional parameter (ϕ) that accounts for violations of the mean-variance assumption in a standard Poisson distribution. For that I used the function glm.cmp in package *mpcmp* (Fung et al., 2020).

Because I had repeated observations from some individuals, I initially considered using mixed-effects models to account for potential non-independence across samples. However, initial tests suggested that the amount of residual variation that was accounted for by individual was in most cases negligible (<1%). I therefore used AICc to evaluate the need for including a random term for individual ID. In most cases, model selection and parsimony suggested that models without a random intercept for individuals were more parsimonious (i.e. had lower AICc values). I therefore opted to use glm's for the analyses, without accounting for potential individual heterogeneity. However, when analyzing the FO for vaccinium the mixed effects model accounting for individual had substantially lower AICc-values (ΔAICc=7.73). In this case, I repeated the analyses presented in the main text by including only the first observation for each bird. All mixed effects models were run using function glmmTMB in package *glmmTMB* (Brooks et al., 2017), with link function and error structure similar to their glm counterparts.

Further, to asses differences in dietary composition among samples from different seasons, sampling year and sex, I calculated distance matrixes for the samples based both on the FO-data and the RRA-data using the distance function from the R-package *phyloseq* (McMurdie & Holmes, 2013). I calculated Jaccard distance matrix for the FO-data and Bray-Curtis distance matrix for the RRA-data (Krebs, 1999). Using the adonis function in the R-package *vegan* (Oksanen et al., 2020), I then performed two separate multivariate analysis of variance

(perMANOVA) models with 999 permutations to test for the effect of season, sampling year and sex on the dietary richness or dietary composition found in the fecal samples. The seasonal variable was here represented by the three time periods covering the sampling season. To test for the assumption of homogeneity of multivariate dispersion, I used the *betadisper* and *permutest* function in R-package vegan with 999 permutations. Finally, to see if any of the detected taxa were strongly associated with any of the predictor variables, I used an indicator taxa analysis from the R-package *indic species* (De Cáceres et al., 2009) based on the RRA data.

All statistical analyses were carried out using R version 4.1.2 (R Development Team 2021).

3.Results

3.1 Final dataset

After taxonomic assignment and removing of false positive ASVs, I identified 18 ASVs as important dietary components across 99 fecal samples from willow ptarmigan (Table 2). Of this 18 ASVs, four were assigned to genus level and 14 to species level. Further analyses were based on these 18 ASVs.

Table 2. Number of occurrences (N.o.) and frequency of occurrence (F꜀%) of important dietary components found across the samples from willow ptarmigan (n = 99).

Family	Species	Functional groups	N.o.	F꜀%
Betulaceae	Betula nana	Trees	95	95.96
Betulaceae	Betula sp.	Trees	95	95.96
Ericaceae	Empetrum nigrum	Dwarf shrubs	80	80.81
Ericaceae	Vaccinium myrtillus	Dwarf shrubs	77	77.78
Ericaceae	Vaccinium sp.	Dwarf shrubs	45	45.45
Betulaceae	Betula pubescens	Trees	42	42.42
Ericaceae	Vaccinium uliginosum	Dwarf shrubs	36	36.36
Ericaceae	Andromeda polifolia	Dwarf shrubs	30	30.3
Ericaceae	Calluna vulgaris	Dwarf shrubs	24	24.24
Ericaceae	Vaccinium vitis-idaea	Dwarf shrubs	19	19.19
Cyperaceae	Eriophorum sp.	Graminoids	19	19.19
Rosaceae	Rubus chamaemorus	Forbs	16	16.16
Rosaceae	Sorbus aucuparia	Trees	9	9.09
Cornaceae	Chamaepericlymenum suecicum	Forbs	8	8.08
Salicaceae	Salix sp.	Trees	7	7.07
Orobanchaceae	Melampyrum pratense	Forbs	7	7.07
Ericaceae	Oxycoccus microcarpus	Dwarf shrubs	5	5.05
Rosaceae	Potentilla erecta	Forbs	3	3.03

3.2 Dietary richness

The most frequently occurring functional groups (based on FO) in the diet were trees (97 %), followed by dwarf shrubs (89 %). Graminoids were present in 20 % of the samples and forbs in 19 % of the samples. Two families, Betulaceae (96 %) and Ericaceae (88 %), were represented in most of the samples. The three most frequent genus across all samples, Betula, Empetrum and Vaccinium, belong to these families. The most frequently occurring ASVs at the species level was *Betula nana* and *Betula spp.*, both found in 96 % of the samples, followed by *Empetrum nigrum* that was found in 81 % of the samples and *Vaccinium myrtillus* in 78 % of the samples.

3.2.1 Seasonal and annual variation in occurrence of most frequent diet items

When modelling frequency of occurrence of Betula as a function of season (Julian date, NDVI or NDSI) and year, I found strongest support for the model including only Julian date (Table 3; Appendix C). The probability that willow ptarmigan included Betula in the diet decreased with Julian date (Figure 4). The models that included Julian date as a descriptor of the seasonal progress outcompeted all models where NDVI or NDSI was included as the seasonal variable (ΔAICc = 6.13 and ΔAICc = 6.62).

Table 3. Candidate models and model statistics for modelling occurrence of Betula in the diet as a function of season (Julian date, NDVI or NDSI) and year. Results from generalized linear models (GLMs) with binary response (1 = detected Betula in sample, 0 = not detected Betula in sample) and logit link function, assuming binomial error distribution.

Response	Model	K	AICc	ΔAICc	AICcWt	CumWt
Betula FO	jDate	2	21.28	0	0.71	0.71
	jDate + Year	4	24.76	3.48	0.13	0.84
	jDate + Year + jDate×Year	6	26	4.72	0.07	0.91
	NDVI	2	27.41	6.13	0.03	0.94
	NDSI	2	27.9	6.62	0.03	0.97
	Intercept	1	28.93	7.65	0.02	0.98
	NDVI + Year	4	30.1	8.82	0.01	0.99
	NDSI + Year	4	30.34	9.06	0.01	1
	NDVI + Year + NDVI×Year	6	33.57	12.29	0	1
	NDSI + Year + NDSI×Year	6	34.49	13.21	0	1

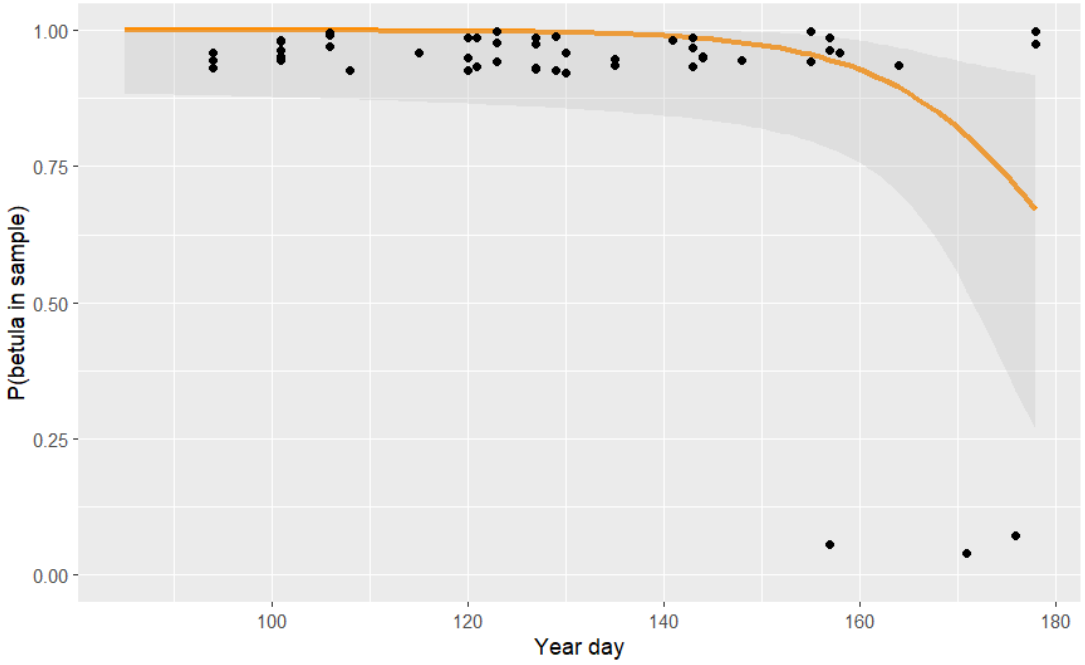

Figure 4. Estimated relationship (solid line) between occurrence of Betula in samples and day of the year. The shaded ribbons represent 95% confidence interval. Function jitter is used for visualization of data points to avoid overlapping points.

When modelling frequency of occurrence of genus Vaccinium as a function of season and year, a model including the main effects Julian date and year was strongest supported by the data (Table 4; Appendix C). The probability of the ptarmigan to include Vaccinium in their diet increased with Julian date, and there was consistently higher occurrence of Vaccinium in 2019 and 2021 than in 2020 (Figure 5). This model received substantially more support than all other models that were examined (all models ΔAICc >13.91). The most supported model showed coinciding result with the model including only the first observation for each bird (Appendix C).

Table 4. Candidate models and model statistics for modelling occurrence of Vaccinium in the diet as a function of season (Julian date, NDVI or NDSI) and year. Results from generalized linear models (GLMs) with binary response (1 = detected Vaccinium in sample, 0 = not detected Vaccinium in sample) and logit link function, assuming binomial error distribution.

Response	Model	K	AICc	ΔAICc	AICcWt	CumWt
Vaccinium FO	jDate + Year	4	65.3	0	1	1
	NDSI + Year + NDSI×Year	6	79.21	13.91	0	1
	NDSI + Year	4	79.96	14.66	0	1
	NDVI + Year + NDVI×Year	6	83.8	18.5	0	1
	jDate	2	83.83	18.53	0	1
	NDVI + Year	4	87.03	21.73	0	1
	NDSI	2	90.29	24.99	0	1
	NDVI	2	95.06	29.76	0	1
	Intercept	1	106.92	41.62	0	1

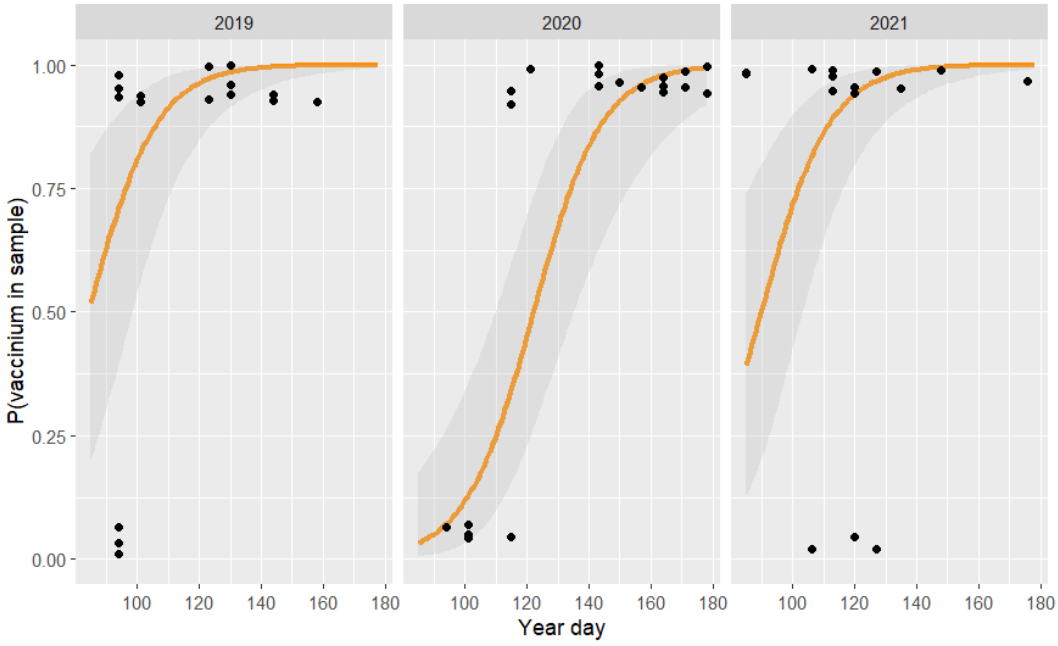

Figure 5. Estimated relationship (solid line) between occurrence of Vaccinium in samples and day of the year. The shaded ribbons represent 95% confidence interval. Function jitter is used for visualization of data points to avoid overlapping points.

The model including the main effects Julian date and year received strongest support when evaluating models that describe seasonal progress in the probability of the ptarmigan to include Empetrum in their diet (Table 5; Appendix C). Occurrence of Empetrum increased with Julian date, and there was less Empetrum included in the diet in year 2020 than in the two other years (Figure 6). However, the model including NDSI (ΔAICc = 1.76) and the model including the main effects NDSI and year (ΔAICc = 1.80), were competitive with the first model, showing that snow cover may as well as day of year explain the probability of the willow ptarmigan to include Empetrum in their diet.

Table 5. Candidate models and model statistics for modelling occurrence of Empetrum in the diet as a function of season (Julian date, NDVI or NDSI) and year. Results from generalized linear models (GLMs) with binary response (1 = detected Empetrum in sample, 0 = not detected Empetrum in sample) and logit link function, assuming binomial error distribution.

Response	Model	K	AICc	ΔAICc	AICcWt	CumWt
Empetrum FO	jDate + Year	4	92.5	0	0.35	0.35
	NDSI	2	94.26	1.76	0.15	0.5
	NDSI + Year	4	94.3	1.8	0.14	0.64
	jDate	2	94.79	2.29	0.11	0.75
	NDVI	2	95	2.5	0.1	0.85
	NDVI + Year	4	95.49	2.99	0.08	0.93
	NDSI + Year + NDSI×Year	6	97.18	4.68	0.03	0.97
	NDVI + Year + NDVI×Year	6	98.51	6.01	0.02	0.99
	Intercept	1	98.86	6.36	0.01	1

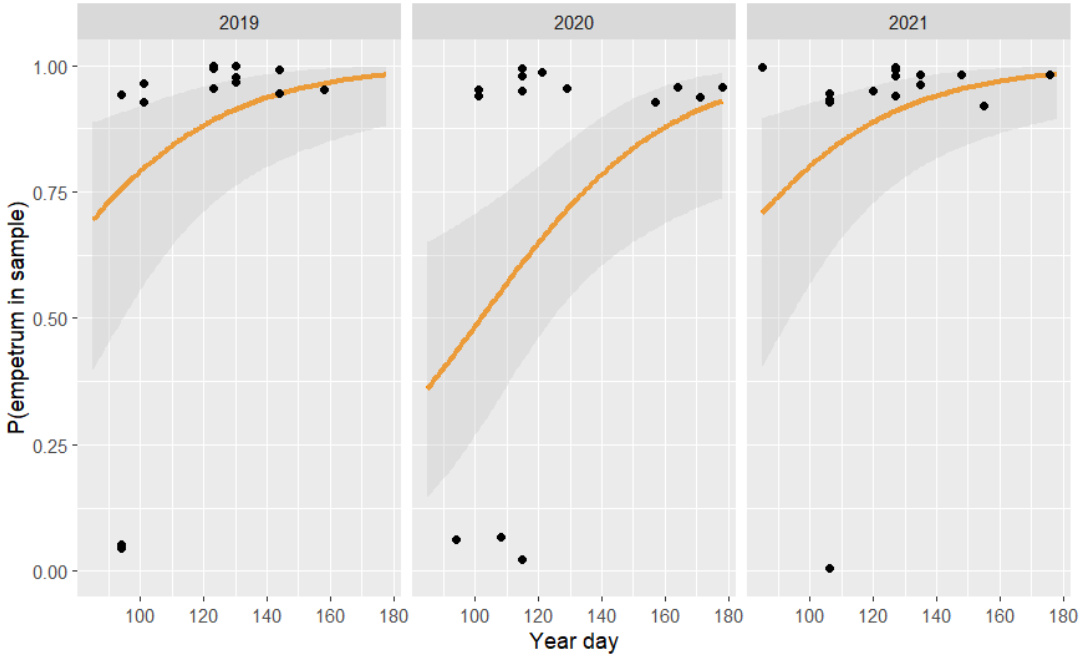

Figure 6. Estimated relationship (solid line) between occurrence of Empetrum in samples and day of the year. The shaded ribbons represent 95% confidence interval. Function jitter is used for visualization of data points to avoid overlapping points

3.2.2 Diversity per sample

From each sample, I identified on average 6.2 ASVs (range: 2-14) at the species level, 2.5 ASVs (range: 1-6) at the family level, and on average 2.3 functional groups (range: 1-4) per sample. When grouping the field period spanning winter to late spring into three time periods covering the sampling season, I identified 12 ASVs at the species level from samples from winter (range pr .sample: 2-9, n=33), 16 ASVs from samples from early spring (range pr. sample: 3-11, n= 36), and 18 ASVs from samples from late spring (range pr. sample: 4-14, n= 30).

When modelling number of species per sample as a function of season (Julian date, NDVI or NDSI), year and sex, I found strongest support for the model including the main effects Julian day, year and sex and the interaction Julian date×year (Table 6, Appendix C). All other models received substantially less support by the data (ΔAICc > 2.84). Model coefficients from the most supported model revealed that the number of species in the willow ptarmigan diet increased with day of the year, but that this seasonal progression differed between years (Appendix C). In addition, females had more diverse diet than males (Figure 7). Similar results were found when modelling number of families and number of functional groups as a function of season, year and sex (Appendix C).

Table 6. Candidate models and model statistics for modelling number of species in the diet as a function of season (Julian date, NDVI or NDSI), year and sex. Results from generalized linear models (GLMs) with count response and log link function, assuming generalized poisson error distribution.

Response	Model	K	AICc	ΔAICc	AICcWt	CumWt
Species pr sample	jDate + Year + Sex + jDate×Year	8	419.74	0	0.72	0.72
	jDate + Year + Sex	6	422.58	2.84	0.17	0.90
	jDate + Year + jDate×Year	7	424.34	4.60	0.07	0.97
	jDate + Year	5	426.05	6.31	0.03	1
	NDSI + Year	5	435.68	15.94	0	1
	NDSI + Year + Sex	6	436.04	16.3	0	1
	NDSI	3	437.57	17.83	0	1
	NDSI + Sex	4	437.83	18.09	0	1
	NDSI + Year + Sex + NDSI×Year	8	437.97	18.23	0	1
	NDSI + Year + NDSI×Year	7	438.37	18.63	0	1
	jDate + Sex	4	438.67	18.93	0	1
	jDate	3	439.66	19.92	0	1
	NDVI + Year	5	442.1	22.36	0	1
	NDVI+ + Year + Sex	6	443.05	23.31	0	1
	NDVI	3	443.96	24.22	0	1
	NDVI + Year + NDVI×Year	7	444.08	24.35	0	1
	NDVI + Year + Sex + NDVI×Year	8	444.45	24.71	0	1
	NDVI + Sex	4	444.86	25.12	0	1
	Intercept	2	467.11	47.37	0	1

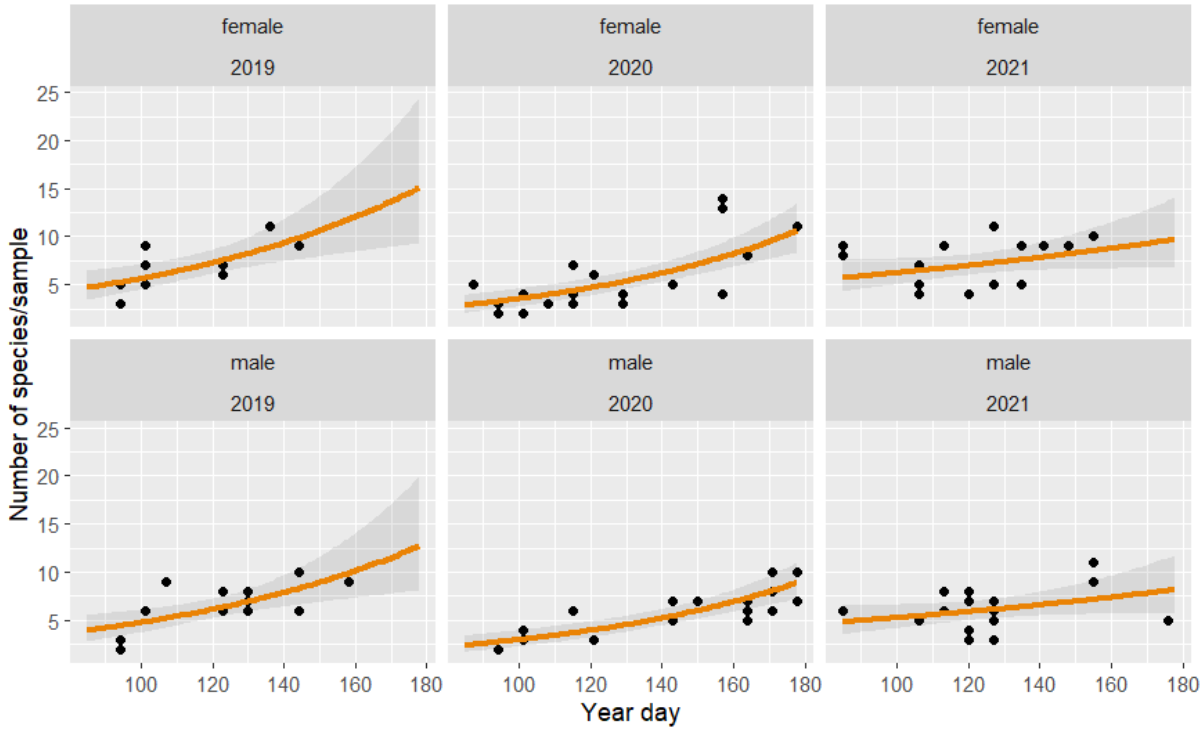

Figure 7. Estimated relationship (solid line) between number of species in samples and day of the year. The shaded ribbons represent 95% confidence interval. Function jitter is used for visualization of data points to avoid overlapping points.

I executed a perMANOVA to test for the effects of the prediction variables (period, year, and sex) on the dietary composition based on FO at the species level. I found strong evidence that there was a difference in diet composition between fecal samples collected from the three different periods and from the three different years (df=4, F=2.07 p=0.009). However, there was no evidence for a difference in dietary composition between males and females across the fecal samples (df=1, F=1.88 p=0.112). The assumption of homogeneity in variance were met for the predictor variables period (p=0.797) and sex (p=0.634). However, the beta dispersal test suggested that the differences observed in dietary composition between years may be inflated due to the lack of homogeneity in variance across those groups (p=0.017).

3.2 Dietary composition

Similar to the dietary richness data, the highest relative read abundance (RRA) of consumed functional groups of plants were trees and dwarf shrubs. Of the analyzed sequences trees represented as much as 54.7 % of the functional groups while dwarf shrub represented 41.8 %. Only 1.8 % of the total reads originated from the group forbs and 0.8 % from the group graminoids. Betulaceae and Ericaceae were by far the most dominant families, making up 54 % and 41.8 % of the total RRA, respectively. The species with highest RRA in family Betulaceae were *Betula sp*. (27.1 % of the total RRA) and *Betula nana* (26.7 % of the total

RRA), while in family Ericaceae, *Vaccinium myrtillus* (20.7 % of the total RRA) and *Empetrum nigrum* (11.3 % of the total RRA) were the species with the highest RRA (Figure 8).

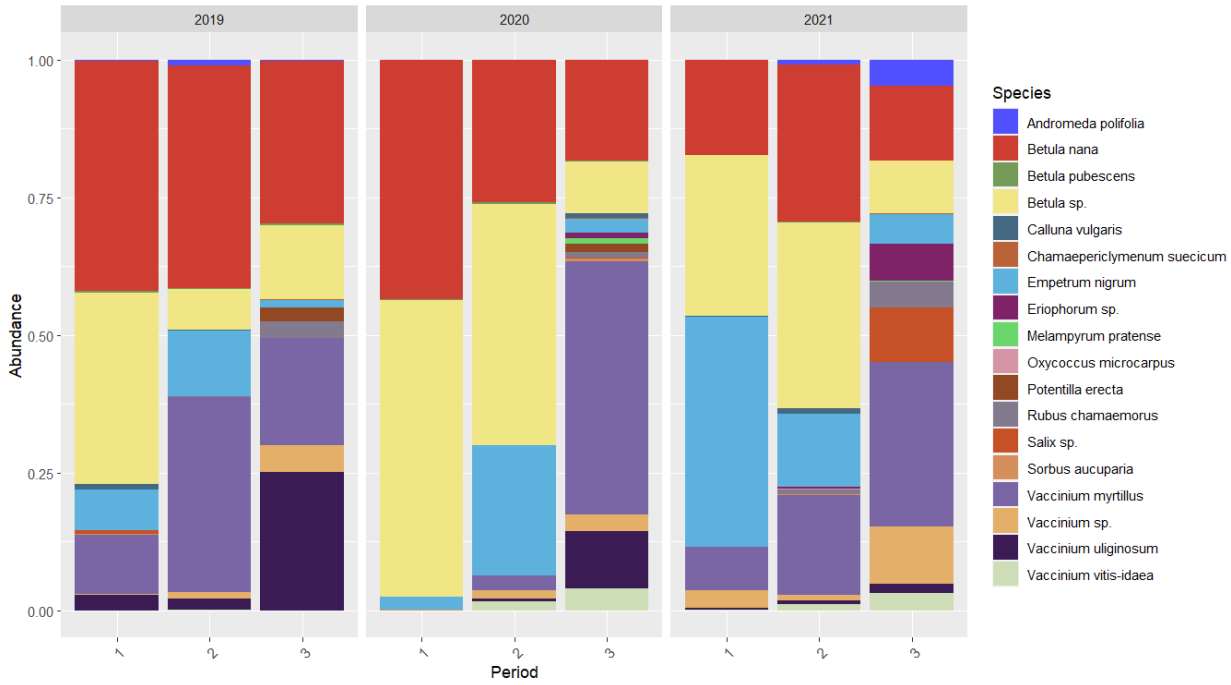

Figure 8. Stacked bar chart showing the proportions of reads assigned to taxonomic range species from the fecal samples collected from willow ptarmigan (n=99). The samples are distributed over the three sampling-years, and the field period is separeted in three equal periods to represent the development from winter to late spring.

3.2.1 Seasonal and annual variation in composition of most abundant diet items

When modelling relative read abundance in the most frequent genus, Betula, as a function of season (Julian date, NDVI or NDSI) and year, three nested models were competitive (Table 7; Appendix C). Thus, the models revealed clear effect of Julian date on the abundance of Betula in samples (model jDate in Table 7), whereas the evidence for effects of year or interaction with year was none conclusive (Table 7). Similar to the dietary richness data, the amount of Betula in the diet decreased with Julian date (Figure 9).

Table 7. Candidate models and model statistics for modelling the proportion of Betula in the diet as a function of season (Julian date, NDVI or NDSI) and year. Results from generalized linear models (GLMs) with continuous response and identity link function assuming Gaussian error distribution.

Response	Model	K	AICc	ΔAICc	AICcWt	CumWt
Betula RRA	jDate + Year + jDate×Year	7	386.59	0	0.39	0.39
	jDate + Year	5	386.65	0.06	0.38	0.78
	jDate	3	387.70	1.12	0.22	1
	NDSI	3	401.00	14.41	< 0.01	1
	NDSI + Year	5	403.86	17.27	< 0.01	1
	NDVI	3	405.23	18.64	< 0.01	1
	NDSI + Year + NDSI×Year	7	405.93	19.34	< 0.01	1
	NDVI + Year	5	408.39	21.80	< 0.01	1
	NDVI + Year + NDVI×Year	7	411.29	24.70	< 0.01	1
	Intercept	2	430.34	43.75	< 0.01	1

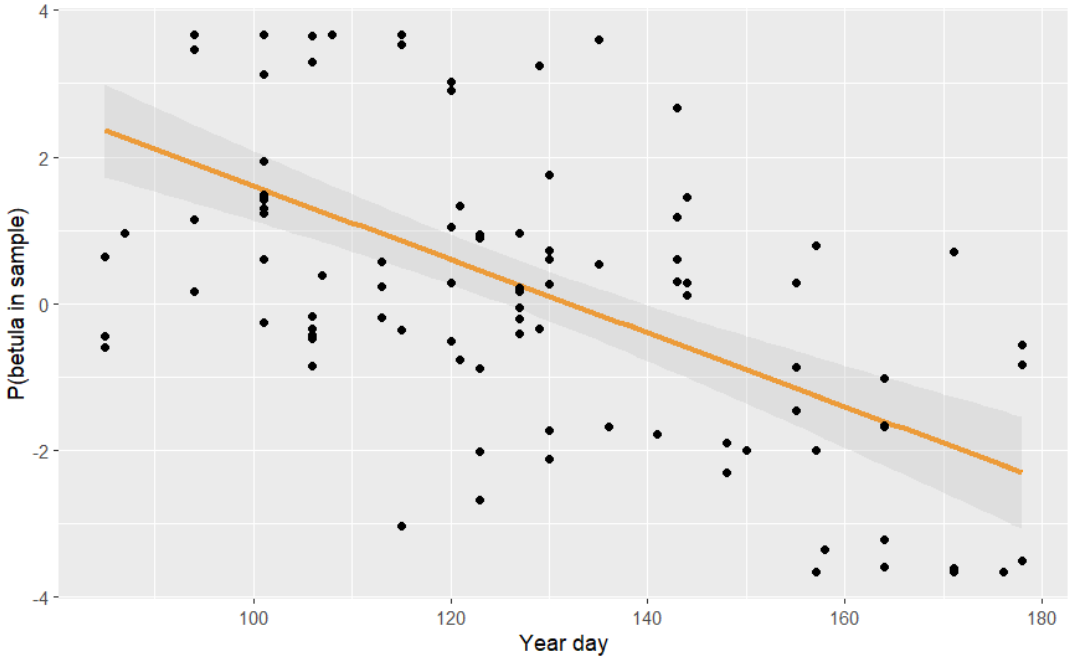

Figure 9. Estimated relationship (solid line) between RRA of Betula in samples and day of the year. The shaded ribbons represent 95% confidence interval.

Modelling the relative read abundance of Vaccinium in the diet as a function of season and year, showed support for the full model including the main effects Julian date and year, and the interaction between them (Table 8; Appendix C). All other models were considered inconclusive (ΔAICc > 7.22). The proportion of vaccinium in the diet increased with Julian date, but the increase in the proportion of Vaccinium and the amount of Vaccinium in the diet were different between the three years (Figure 10).

Table 8. Candidate models and model statistics for modelling the proportion of Vaccinium in the diet as a function of season (Julian date, NDVI or NDSI) and year.

Response	Model	K	AICc	ΔAICc	AICcWt	CumWt
Vacc. RRA	jDate + Year + jDate×Year	7	349.13	0	0.97	0.97
	jDate + Year	5	356.35	7.22	0.03	1
	jDate	3	360.99	11.86	0	1
	NDSI	3	379.68	30.55	0	1
	NDSI + Year + NDSI×Year	7	380.78	31.66	0	1
	NDSI + Year	5	383.67	34.54	0	1
	NDVI	3	386.18	37.05	0	1
	NDVI + Year +NDVI×Year	7	389.01	39.88	0	1
	NDVI + Year	5	390.02	40.89	0	1
	Intercept	2	409.21	60.08	0	1

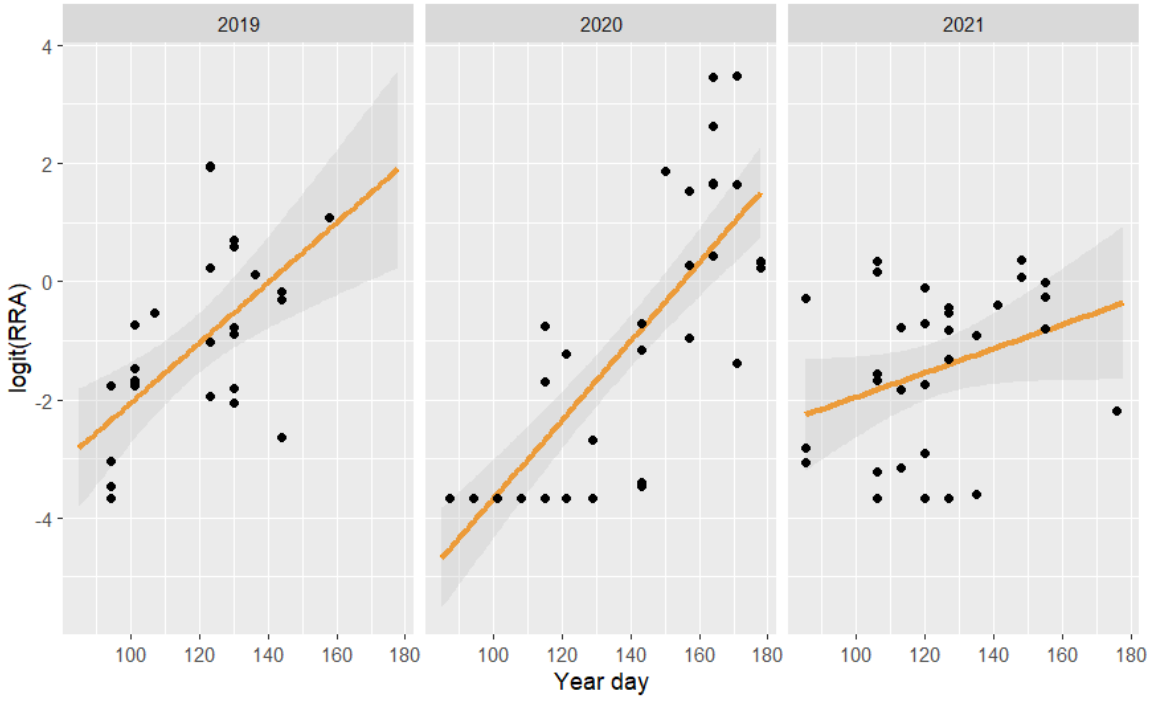

Figure 10. Estimated relationship (solid line) between RRA of Vaccinium in samples and day of the year. The shaded ribbons represent 95% confidence interval.

When modelling relative read abundance of Empetrum in the diet as a function of season and year, three models were competitive (Table 9; Appendix C). The first two models were nested and revealed an effect of Julian date on the abundance of Empetrum in samples (model jDate in Table 9), whereas the evidence for effects of year or interaction with year was not conclusive (Table 9). Due to higher AICc (ΔAICc < 1.98) and low AICcWt (<0.14) the third model was considered to explain only a minor part of the variation in abundance of Empetrum in the samples. The amount of Empetrum in the diet was found to decrease with Julian date (Figure 11).

Table 9. Candidate models and model statistics for modelling the proportion of Empetrum in the diet as a function of season (Julian date, NDVI or NDSI) and year. Results from generalized linear models (GLMs) with continuous response and identity link function assuming Gaussian error distribution

Response	Model	K	AICc	ΔAICc	AICcWt	CumWt
Empetr. RRA	jDate	3	326.69	0	0.34	0.34
	jDate + Year + jDate×Year	7	328.54	1.85	0.14	0.48
	NDSI	3	328.67	1.98	0.13	0.6
	jDate + Year	5	328.77	2.08	0.12	0.72
	NDVI	3	328.93	2.24	0.11	0.84
	Intercept	2	330.5	3.81	0.05	0.89
	NDSI + Year	5	330.51	3.82	0.05	0.94
	NDVI + Year	5	330.74	4.05	0.05	0.98
	NDSI + Year + NDSI×Year	7	333.78	7.09	0.01	0.99
	NDVI + Year + NDVI×Year	7	334.22	7.53	0.01	1

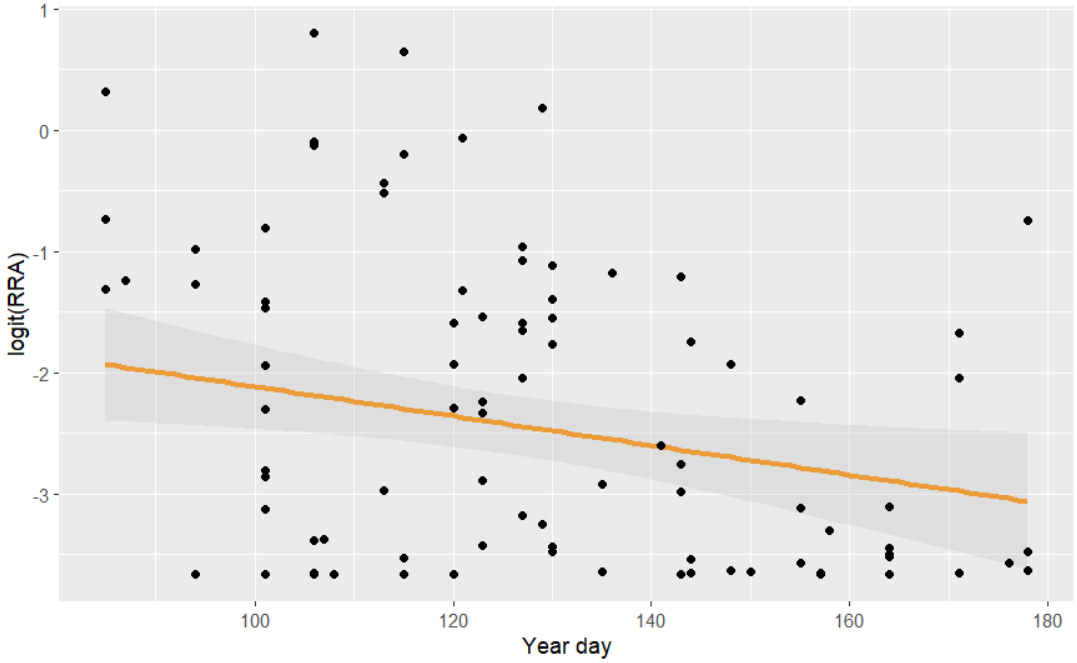

Figure 11. Estimated relationship (solid line) between RRA of Empetrum in samples and day of the year. The shaded ribbons represent 95% confidence interval.

To asses differences in dietary composition between years, sex and period, I used perMANOVA. I found strong evidence that the dietary composition differed between year and period (df=4, F=3.06, p=0.002) (Figure 8). However, there was no evidence for a difference between males and females (df=1, F=1.25, p=0.28). The assumption of homogeneity in variance was met for predictor variables sex (p=0.31) and year (p=0.41), but not for period (p=0.001). This might suggest that the reported effect might be inflated.

From the indicator taxa analysis based on the three equal time-periods, *Betula sp.*, *Betula nana* and *Empetrum Nigrum* were species strongly associated with winter and early spring ($p < 0.05$), while *Vaccinium myrtillus* was strongly associated with the early and late spring ($p < 0.05$). The analysis further showed that six species were strongly associated with late spring: *Vaccinium uliginosum, Eriophorum sp., Rubus chamaemorus, Vaccinium sp., Melampyrum pratense* and *Potentilla erecta* ($p < 0.05$). Two species, *Betula nana* and *Betula pubescens*, were strongly associated with sampling year 2019 ($p < 0.05$), and the species *Vaccinium myrtillus* was strongly associated with samples collected from males ($p < 0.05$).

4.Discussion

Trough metabarcoding of 99 fecal samples from willow ptarmigan I identified 18 important diet components in the willow ptarmigan diet during the transition from winter to late spring. I found that species from the functional groups of trees and dwarf shrubs dominated the diet at this time of year, and within these groups there were three genera which constituted the most important diet components: Betula, Vaccinium and Empetrum. These genera differed in temporal abundance and occurrence throughout the season. Partly in line with my first prediction, I found that the winter diet of willow ptarmigan was dominated by Betula species, and that elements of nutritious field vegetation in the diet increased as the spring progressed. While Betula constituted a declining part of the diet throughout the spring, occurrence and abundance of dwarf shrubs, forbs and graminoids increased. However, dwarf shrubs, especially Empetrum, also constituted an important part of the winter diet of willow ptarmigan. I found that diet richness and composition varied across time, but in contrast to my second prediction, this dietary variation was to a larger extend explained by Julian date than by snow cover (NDSI) and vegetation phenology (NDVI) in the study area. There was some support for a sex-based difference in diet: I found that females had a more diverse diet than males, but no support for a difference in diet composition between the sexes across all fecal samples.

4.1 The most important dietary components of willow ptarmigan diet and its variation across time

Herbivore species living in seasonal environments are expected to make foraging choices that track the timing of spring vegetation growth and the seasonal availability of nutritious plants (Espunyes et al., 2022). For willow ptarmigan, after a harsh winter, access to nutritious field vegetation in spring is important to acquire energy reserves for the breeding period (Moss et al., 1975; Brittas, 1988). A main finding of this study is that willow ptarmigan has a gradual shift in diet composition and richness in the transition from winter to late spring. As predicted, they switch from a relatively narrow winter diet to broader spring diet with increasing elements of nutritious field vegetation. This is expected given that vegetation is subject to seasonal effects and is consistent with previous studies (Brittas, 1988; Pulliainen & Tunkkari, 1991). During winter (time period 1) the functional group trees, with Betula as the dominant genus, is the most important diet component along with varying abundance of dwarf shrubs. Throughout the spring, the relative importance of Betula declines. Of the three most important genera in the diet, Betula is also the only genus that do not show an inter-annual change in either FO or RRA. Inter-annual variations in the arrival of spring and snow cover therefore do not seem to affect

willow ptarmigan intake of Betula, and this is probably related to the types of food items that is most available in the winter when snow depth is at its highest. Several studies from other areas have pointed out willow (*Salix spp*.) as the preferred winter food for willow ptarmigan (Brittas, 1988; Elson et al., 2007; Hakkarainen et al., 2007). However, willow is much less abundant than birch in my study area and largely snow-covered during the winter.

Although a narrow winter diet is documented in previous studies, metabarcoding provides a better taxonomic resolution and thus provide the possibility of a broader identification of species that can be difficult to distinguish from each other under traditional methods (Sousa et al., 2019). As many as 12 of 18 identified ASVs were also detected in the winter diet, indicating that willow ptarmigan takes advantage of a wider winter diet than previously thought. This richness is probably due to access to last year's plant production in areas where the snow cover is sparse. Whereas Brittas (1988) in a six-year study in Sweden only detected 0.5 % dwarf shrubs in late winter diet, and only blueberries, I have shown that dwarf shrubs, and especially crowberry, seems to be important elements in the willow ptarmigan winter diet. Indicator taxa analysis shows that crowberry is strongly associated with the first field period, and for example in 2021, Empetrum and Vaccinium accounted for over 50 % of the total RRA from the samples from winter (Figure 8). Crowberry grows on exposed ridges in the mountains, which can often be without snow cover. Willow ptarmigan thus has the opportunity to use both evergreen plant parts and last-season berries from crowberry as a food resource throughout the winter. Along with crowberry, species in genus Vaccinium are also important elements in the winter diet. However, in contrast to the abundance of Empetrum in the diet which decreased during the season, the abundance of species in vaccinium in the diet increased. The importance of bilberry shoots as spring food was highlighted by Brittas (1988) and Pulliainen & Tunkari (1991) and is also confirmed in this study. However, my findings propose that willow ptarmigan utilize a wider range of vaccinium species in spring than documented in earlier studies.

In early spring (time period 2), plants from the functional group dwarf shrubs are just as abundant in the diet as trees, and in late spring (time period 3) dwarf shrubs dominate the diet along with increasing elements of graminoids and forbs. For all three periods together, only a small percentage of the plants in the diet were represented by forbs and graminoids. Only 1.8 % of the total reads originated from the group of forbs and 0.8 % from the group of graminoids. This is probably because sampling took place in late winter and spring, and species from these groups will probably have an increasing abundance in the diet over the summer. As expected, the proportion of forbs and graminoids in the diet increased throughout spring. Although these

functional groups only were present in small proportions, previous studies have shown that they are of great importance for the ptarmigan. For example, Brittas (1988) found that even though cotton grass was a minor component in the diet of willow ptarmigan in spring, a positive correlation between food digestibility and the percentage of cotton grass flowers in the diet was found. The shift in feeding process from winter to late spring is related both to seasonality and diet quality. Due to reduced snow cover in spring willow ptarmigan will have more diets items available and by shifting from a diet dominated by trees to a broader diet with dwarf shrubs, graminoids and forbs, willow ptarmigan obtains a more nutritious diet. The consumption of the different diet items shows an inter-annual variation and this results in a variation in diet composition and richness between years. Due to the fact that spring diet affects breeding success (Moss & Watson, 1984; Brittas, 1988), this variation could affect willow ptarmigan condition and fitness.

4.2. The variation in diet richness and composition as explained by the abiotic factors vegetation phenology and snow cover

In my analyses the variation across time in diet composition and diet richness is in general better explained by Julian date than by snow cover and vegetation phenology measured by NDVI and NDSI respectively. Previous studies of ptarmigan diet have found that the availability and quality of diet in spring is associated with the timing of snowmelt and proposed that snowmelt affect plant species availability and phenology and thereby the diet composition of ptarmigan (Garcia-Gonzales et al., 2016). My results might, however, indicate that on a finer temporal scale there may also be other factors that influence willow ptarmigan choice of diet than just availability. Even though NDVI and NDSI give us good indices on the amount of green biomass and snow cover in the study area, the indices don't necessarily represent the areas where the ptarmigans are found. Willow ptarmigan does not have equal distribution across a landscape, and within their range, individual birds generally prefer habitats with a high density of food and cover from predators (Erikstad, 1985). Some factors related to life history traits of the bird also affect the species distribution across a landscape in the transition from winter to spring, and thus also can affect the diet composition. First, males in spring defend breeding territories of 2–12 ha (Pedersen, 1984). After mating with a male in late winter / early spring the female feeds almost solely within the male territory (Lance, 1983), thus location and size of territory will affect available diet items for both sexes. Second, foraging strategy during snow melt proves to be a compromise between food quality and optimal cover. The willow ptarmigan is an important prey in the alpine ecosystem, and therefore display well-developed antipredator strategies. In spring, willow ptarmigan change plumage from a white winter plumage to a

browner summer plumage. At the same time, snow melting in the breeding habitat creates a mosaic of white and dark areas, and it has been shown that during the snow melt ptarmigan can choose to forage in areas that give them camouflage rather than optimal nutrition (Savory, 1983; Steen et al., 1992). An earlier snowmelt in the alpine areas can lead to a mismatch between the ptarmigan moulting and the presence of snow (Melin et al., 2020), and this may cause the ptarmigan to seek areas with snow during foraging even though green biomass is available. Although global warming is assumed to lead to an earlier and longer access to green nutrient-rich plant material, it does not necessarily lead to a temporal change in willow ptarmigan diet composition. Apparently there are also other factors than availability of fresh vegetation that influence willow ptarmigan during foraging.

In support of my prediction, I found that there is a variation in diet composition between years, but I could not find support that this inter-annual variation is explained by either NDVI or NDSI. One reason for this could be that large parts of the plant species in the willow ptarmigan diet show cyclicity and will therefore have an inter-annual and interseasonal variation in both nutrient content and abundance (Brittas, 1988). Since the willow ptarmigan has the ability to be selective during foraging (Pulliainen & Salo, 1973), this cyclicity will affect what the birds choose to eat as well as what they have access to. After snowmelt alpine plants experience a rapid growth period associated with an increase in nutrients and digestibility (Körner, 1999). There will be variations between different plant species as to when they reach their peak in nutrition, and in a study of Pyrenean rock ptarmigan (*Lagopus muta pyrenaica*) Garcia-Gonzales et al. (2016b) found that diet quality is more dependent on the phenological stage of food components than their floristic composition. The timing of snow melt differs between years, and this affects plant phenology and species peak in nutrition. This, in addition to plant cyclicity, can possibly result in inter-annual variations in willow ptarmigan diet composition.

Although Julian date outperformed NDVI and NDSI in predicting the temporal variation in diet, the results coincide with NDVI and NDSI indices in a broader view. Willow ptarmigan had less abundance of blueberries and less likelihood of including crowberry in the diet in years with longer temporal persistence of snow cover and later spring onset. Likewise, Betula species were more abundant in the winter diet in years with higher NDSI values and this indicates little availability of other more nutritious plants due to snow cover. In contrast, blueberry was found to be more abundant in the diet during winter in years with lower NDSI values and higher NDVI values, following the expectations that willow ptarmigan has earlier access to field vegetation in years with early spring.

NDVI has been established as a crucial tool for assessing vegetation phenology and primary productivity, but there are also limitations in use of satellite-based ecological data (Pettorelli et al., 2011). Dietary variation across time in willow ptarmigan could not be explained by snow cover and vegetation phenology in this study. This could be due to intrinsic factors related to the ptarmigan, extrinsic factors related to vegetation cyclicity and phenology, or it could be due to limitations with the measurement method, in this case NDVI and NDSI. The need to validate results from NDVI with independent data has been highlighted (Pettorelli et al., 2011). In this study, methods for validating the results from NDVI and NDSI with local data could with advantage have been developed. This could have led to a further explanation of how snow cover and vegetation phenology affect the willow ptarmigan diet in the transition from winter to spring.

4.3 Sex-based differences in willow ptarmigan diet

I did not detect a difference in diet composition between male and female willow ptarmigan. This is in accordance with previous studies from Fennoscandia (Brittas, 1988; Pulliainen & Tunkari). However, in my study females were found to have a more diverse diet than males. Metabarcoding can give better taxonomic resolution and identify a wider breadth of diet items (Valentini et al., 2009), so this result could have been overlooked in studies using other methods. In a study from Canada, Elson et al. (2007) showed that female willow ptarmigan feed on higher nutritive quality foods than males in winter and prior to egg lying, and it is conceivable that the difference in diet richness supports the fact that females are more selective and search for plants that contain higher nutritive foods during the spring than males. However, the indicator taxa analyses showed that blueberry was strongly associated with males. I have not found support for this difference in previous studies, but Pulliainen & Tunkari (1991) suggest that sugar rich berries may be an important dietary component for males during the defense of their territories. The detected difference could also be due to different habitat selection between the sexes, and further studies are required to answer this question.

4.4 Metabarcoding of fecal pellets in dietary studies of herbivore birds

This study demonstrates that metabarcoding of fecal pellets is suited to estimate the dominant food plants in the diet of willow ptarmigan. This non-invasive method is more sensitive to detect a larger variety of diet items than traditional methods as examination of crop material and observation studies (Valentini et al., 2009; Chua et al., 2021; Fujii et al., 2021). Collection of fecal pellets represents a field method that is easy to implement and collecting fecal samples over temporal and spatial scales provides the opportunity to assess factors that affect diet

composition. The molecular methods applied in this study also have some limitations in assessing the diet via analysis of fecal pellets. First, 21 of 141 samples failed to amplify during the PCR amplification process, and this may be related to DNA degradation due to the time between defecation and sampling (Ando et al., 2020). This highlights the importance of sampling pellets as fresh as possible. Further, the characteristics that make metabarcoding highly sensitive to detect species, also make the method vulnerable to potential contamination and detection of false positive elements in the diet (Ando et al., 2020). In this study, 27 detected species were found to be unlikely for the study area, and to exclude false positives in the result all species that had the same or less total sequence amount than the false positive species with the highest total amount were removed. This meant that 68 of 86 ASVs were excluded from further analyzes and results. The removed species represented a very small proportion of the sequences and thus were considered not to be important dietary components. However, it is likely that some of the species that were removed were actually included in the ptarmigan diet. For example, alpine bistort (*Bistorta vivipara*) which has been reported to be a preferred plant by other species of ptarmigan (Unander et al., 1985), was removed in this operation. The list of documented food items can thus be considered as a minimum of their entire diet composition.

5. Conclusion

Through this study I have shown that metabarcoding of fecal pellets is a successful method for gaining insight into the diet of willow ptarmigan, and that this method, in combination with remote sensing tools, can provide the opportunity to investigate how seasonal variables affect the diet over temporal and spatial scales. The willow ptarmigan diet composition and richness were found to vary both seasonally and inter-annually. Snow cover and vegetation phenology, as measured by NDVI and NDSI respectively, were however not found to explain the variation in diet. This could indicate that although snow cover and vegetation phenology affect the availability and abundance of plant food in ptarmigan habitats, other intrinsic and extrinsic factors can contribute to explaining the temporal variation in ptarmigan diet. However, limitations in use of satellite-based data could also have affected the results, and validation through local methods is therefore needed. Female willow ptarmigan was found to have a more diverse diet than males, while I found no sex-based difference in the diet composition across all fecal samples.

Metabarcoding of fecal pellets is a non-invasive method that provides information with a greater taxonomic resolution than traditional methods in dietary studies. Through simple field methodology with sampling of fecal pellets, it is now possible to conduct dietary studies at different temporal and spatial scales. I highlight the need for further metabarcoding studies of willow ptarmigan diet. By sampling fresh pellets throughout the year, one can document the most important diet components throughout the seasons and have the opportunity to assess which factors influence the diet through the annual cycle. Further, sampling in different study areas gives the opportunity to expand the understanding of which factors affect diet over larger spatial scales. This will provide useful information in further management of willow ptarmigan. Global warming will affect vegetation phenology and change abundance and distribution of mountain species. To understand and predict how these changes affect the life history traits of willow ptarmigan it is essential to have knowledge about the diet and factors affecting the diet throughout the year. Through knowledge about inter-annual variability in diet and individual dietary preferences it will further be possible to link this information to variation in life history traits as clutch size, breeding success and survival, and asses how diet in a changing climate can affect willow ptarmigan condition and fitness.

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Appendix

Appendix A

Feltskjema for grønningsprosjektet, Lifjellet

Dato: Navn på alle feltarbeidere: Evt. kommentar:

Prøvenavn (4 sifre fra lapp med unik ID)	Rutenummer (rute 1-7, hvis innenfor en rute)	UTM (hvis mellom ruter)	Kryss for at rutenummer (UTM hvis mellom ruter) og dato er skrevet på glasset, og lapp med unik ID er klistret på glasset	Evt. merknad

Figure A1: Field form

Appendix B

Table 1. 86 amplicon sequence variants (ASVs) found across the samples from willow ptarmigan (n = 99). Taxa sums represents total number of reads of each ASV across all fecal samples.

Family	Genus	Species	Taxa sums
Betulaceae	Betula	Betula nana	4436903
Betulaceae	Betula	Betula sp.	3940869
Ericaceae	Vaccinium	Vaccinium myrtillus	2828659
Ericaceae	Empetrum	Empetrum nigrum	1649993
Ericaceae	Vaccinium	Vaccinium uliginosum	470267
Ericaceae	Calluna	Calluna vulgaris	62770
Ericaceae	Vaccinium	Vaccinium sp.	264933
Ericaceae	Andromeda	Andromeda polifolia	67077
Ericaceae	Vaccinium	Vaccinium vitis-idaea	144917
Cyperaceae	Eriophorum	Eriophorum sp.	72241
Ericaceae	Arctous	Arctous alpina	323
Rosaceae	Rubus	Rubus chamaemorus	80571
Salicaceae	Salix	Salix sp.	70053
Rosaceae	Potentilla	Potentilla erecta	45702
Orobanchaceae	Melampyrum	Melampyrum pratense	24794
Betulaceae	Betula	Betula pubescens	23512
Rosaceae	Sorbus	Sorbus aucuparia	18733
Cornaceae	Chamaepericlymenum	Chamaepericlymenum suecicum	5513
Ericaceae	Oxycoccus	Oxycoccus microcarpus	3027
Geraniaceae	Geranium	Geranium sylvaticum	1323
Poaceae	Agrostis	Agrostis gigantea	1650
Brassicaceae	Arabidopsis	Arabidopsis thaliana	1108
Brassicaceae	Conringia	Conringia orientalis	953
Fabaceae	Lathyrus	Lathyrus latifolius	2013
Apiaceae	Anthriscus	Anthriscus sylvestris	927
Poaceae	Poa	Poa sp.	607
Fabaceae	Medicago	Medicago sativa	515
Brassicaceae	Brassicaceae_sp.		796

Betulaceae	Alnus	Alnus incana	402
Brassicaceae	Brassica	Brassica oleracea botrytis	423
Juniperus	Juniperus_sp.		629
Picea	Picea_sp.		383
Cucurbitaceae	Cucumis	Cucumis sativus	366
Poaceae	Festuca	Festuca sp.	731
Poaceae	Alopecurus	Alopecurus myosuroides	326
Poaceae	Elymus	Elymus sp.	384
Apiaceae	Aegopodium	Aegopodium podagraria	297
Poaceae	Avenella	Avenella flexuosa	302
Poaceae	Dactylis	Dactylis glomerata	403
Droseraceae	Drosera	Drosera anglica	276
Ranunculaceae	Ranunculus	Ranunculus sardous	275
Asteraceae	Scorzoneroides	Scorzoneroides autumnalis	666
Orobanchaceae	Melampyrum	Melampyrum sylvaticum	597
Brassicaceae	Brassica	Brassica sp.	346
Rosaceae	Sorbus	Sorbus sp.	393
Fagaceae	Fagus	Fagus sylvatica	84
Betulaceae	Corylus	Corylus avellana	116
Rosaceae	Prunus	Prunus persica	116
Poaceae	Pseudoroegneria	Elytrigia repens	115
Asteraceae	Solidago	Solidago sp.	146
Onagraceae	Epilobium	Epilobium sp.	107
Asteraceae	Senecio	Senecio vulgaris	38
Poaceae	Secale	Secale cereale	93
Pinus	Pinus_mugo		154
Asteraceae	Cyclachaena	Cyclachaena xanthiifolia	85
Poaceae	Agrostis	Agrostis sp.	170
Ericaceae	Ericaceae_sp.		72
Malvaceae	Sida	Sida rhombifolia	31
Salicaceae	Populus	Populus sp.	126
Salicaceae	Salix	Salix alaxensis alaxensis	165
Betulaceae	Betula	Betula pendula	34

Pinus	Pinus_sp.		121
Asteraceae	Ambrosia	Ambrosia psilostachya	84
Droseraceae	Drosera	Drosera rotundifolia	56
Hylocomiaceae	Pleurozium	Pleurozium schreberi	33
Fagaceae	Quercus	Quercus rubra	40
Poaceae	Poaceae_sp.		29
Lamiaceae	Lamium	Lamium hybridum	22
Chenopodiaceae	Spinacia	Spinacia oleracea	20
Plagiotheciaceae	Plagiothecium	Plagiothecium sp.	20
Cyperaceae	Carex	Carex pauciflora	18
Cyperaceae	Cyperaceae_sp.		16
Poaceae	Digitaria	Digitaria ischaemum	14
Poaceae	Triticum	Triticum sp.	14
Cyperaceae	Eriophorum	Eriophorum angustifolium	13
Polygonaceae	Bistorta	Bistorta vivipara	11
Apiaceae	Torilis	Torilis japonica	10
Amaryllidaceae	Allium	Allium cepa	10
Fagaceae	Quercus	Quercus sp.	9
Salicaceae	Salix	Salix alaxensis	9
Orchidaceae	Neottia	Neottia cordata	6
Aneuraceae	Aneura	Aneura pinguis	6
Anastrophyllaceae	Anastrophyllaceae_sp.		6
Brachytheciaceae	Sciuro-hypnum	Sciuro-hypnum reflexum	4
Poaceae	Lolium	Lolium sp.	4
Brachytheciaceae	Sciuro-hypnum	Sciuro-hypnum sp.	4

Appendix C

Dietary Richness

Betula:

Table C1. Parameter estimates for the best model when modelling occurrence of Betula in the diet as a function of season (Julian date, NDVI or NDSI) and year.

Model: Betula FO ~ jDate	**Estimate**	**Std.Error**
Intercept	19.031	± 8.525
jDate	-0.103	± 0.051

Vaccinium:

Table C2. Parameter estimates for the best model when modelling occurrence of Vaccinium in the diet as a function of season (Julian date, NDVI or NDSI) and year.

Model: Vaccinium FO ~ jDate + Year	**Estimate**	**Std.Error**
Intercept	-7,665	± 2.288
jDate	0.091	± 0.022
Year 2	-3.45	± 0.948
Year 3	-0.508	± 0.909

Table C3. Candidate models and model statistics for modelling occurrence of Vaccinium in the diet as a function of season (Julian date, NDVI or NDSI) and year, when only the first observation of each bird is included. Results from generalized linear models (GLMs) with binary response (1 = detected Vaccinium in sample, 0 = not detected Vaccinium in sample) and logit link function, assuming binomial error distribution.

Response	**Model**	**K**	**AICc**	**ΔAICc**	**AICcWt**	**CumWt**
Vaccinium FO	jDate + Year	4	35.82	0	0.54	0.54
	jDate + Year + jDatexYear	6	36.27	0.45	0.43	0.98
	NDSI + Year	4	42.97	7.14	0.02	0.99
	NDSI + Year + NDSI×Year	6	45.78	9.96	0	1
	NDVI + Year	4	48.34	12.52	0	1
	NDVI + Year + NDVI×Year	6	49.13	13.3	0	1
	jDate	2	64.92	29.1	0	1
	NDSI	2	65.5	29.68	0	1
	NDVI	2	66.49	30.67	0	1
	Intercept	1	72.24	36.42	0	1

Table C4. Parameter estimates for the model including only the first observation of each bird when modelling occurrence of Vaccinium in the diet as a function of season (Julian date, NDVI or NDSI) and year.

Model: Vaccinium FO ~ jDate + Year	**Estimate**	**Std.Error**
Intercept	-7.613	± 3.542
jDate	0.100	± 0.034
Year 2	-5.678	± 1.609
Year 3	-0.600	± 1.358

Empetrum:

Table C5. Parameter estimates for the best models when modelling occurrence of Empetrum in the diet as a function of season (Julian date, NDVI or NDSI) and year.

Model: Empetrum FO ~ jDate + Year	**Estimate**	**Std.Error**
Intercept	-2.058	± 1.463
jDate	0.034	± 0.012
Year 2	-1.391	± 0.694
Year 3	-0.06	± 0.777
Model: Empetrum FO ~ NDSI	**Estimate**	**Std.Error**
Intercept	2.942	± 0.852
NDSI	-2.367	± 1.146
Model: Empetrum FO ~ NDSI + Year	**Estimate**	**Std.Error**
Intercept	3.237	± 0.924
NDSI	-2.455	± 1.15
Year 2	-0.823	± 0.671
Year 3	0.441	± 0.783

Diversity per sample:

Table C6. Parameter estimates for the best model when modelling number of species in the diet as a function of season (Julian date, NDVI or NDSI), year and sex.

Model: Nr.species ~ jDate * Year + Sex	**Estimate**	**Std.Error**
Intercept	0.474	± 0.366
jDate	0.013	± 0.003
Year 2	-0.622	± 0.460
Year 3	0.782	± 0.477
Sex male	-0.173	± 0.064
jDate:Year 2	0.002	± 0.004
jDate:Year 3	0.007	± 0.004

Table C7. Candidate models and model statistics for modelling number of families in the diet as a function of season (Julian date, NDVI or NDSI), year and sex. Results from generalized linear models (GLMs) with count response and log link function, assuming generalized poisson error distribution.

Response	**Model**	**K**	**AICc**	**ΔAICc**	**AICcWt**	**CumWt**
Fam. pr sample	jDate + Year+ Sex	6	270.24	0	0.21	0.21
	NDSI + Sex	4	270.51	0.27	0.18	0.39
	NDSI + Year	5	270.57	0.33	0.17	0.56
	NDSI + Year + Sex	6	270.63	0.39	0.17	0.73
	NDSI	3	271.1	0.86	0.13	0.86
	jDate + Year	5	272.95	2.71	0.05	0.92
	jDate + Year + Sex + jDate×Year	8	274.77	4.53	0.02	0.94
	NDSI + Year + NDSI×Year	7	275.12	4.88	0.02	0.96
	NDSI + Year + Sex + NDSI×Year	8	275.25	5.01	0.02	0.97
	jDate + Year + jDate×Year	7	277.41	7.17	0.01	0.98
	jDate + Sex	4	277.46	7.22	0.01	0.98
	NDVI	3	278.38	8.14	0	0.99
	NDVI + Year	5	278.39	8.15	0	0.99
	NDVI + Sex	4	278.65	8.41	0	0.99
	jDate	3	279.06	8.82	0	1
	NDVI+ Year + Sex	6	279.12	8.88	0	1
	NDVI + Year + NDVI×Year	7	282.23	11.99	0	1
	NDVI + Year + Sex + NDVI×Year	8	282.88	12.64	0	1
	Intercept	2	308.69	38.45	0	1

Table C8. Candidate models and model statistics for modelling number of functional groups in the diet as a function of season (Julian date, NDVI or NDSI), year and sex. Results from generalized linear models (GLMs) with count response and log link function, assuming generalized poisson error distribution.

Response	Model	K	AICc	ΔAICc	AICcWt	CumWt
FG pr sample	jDate + Year+ Sex	6	194.38	0	0.8	0.8
	jDate + Year + Sex + jDate×Year	8	198.76	4.38	0.09	0.89
	jDate + Year	5	199.45	5.07	0.06	0.96
	NDSI + Year + Sex	6	203.16	8.78	0.01	0.97
	jDate + Sex	4	203.2	8.82	0.01	0.98
	NDSI + Year	5	203.71	9.33	0.01	0.98
	jDate + Year + jDate×Year	7	203.89	9.51	0.01	0.99
	NDSI + Sex	4	204.88	10.51	0	0.99
	NDSI	3	206.26	11.88	0	1
	jDate	3	206.78	12.4	0	1
	NDSI + Year + Sex + NDSI×Year	8	207.3	12.92	0	1
	NDSI + Year + NDSI×Year	7	207.79	13.41	0	1
	NDVI + Year	5	213.51	19.13	0	1
	NDVI + Year + Sex	6	213.85	19.47	0	1
	NDVI + Sex	4	214.65	20.27	0	1
	NDVI	3	214.89	20.51	0	1
	NDVI + Year + NDVI×Year	7	216.41	22.03	0	1
	NDVI + Year + Sex + NDVI×Year	8	216.67	22.29	0	1
	Intercept	2	244.75	50.37	0	1

Diet composition

Betula:

Table C9. Parameter estimates for the best model when modelling the proportion of Betula in the diet as a function of season (Julian date, NDVI or NDSI) and year.

Model: Betula RRA ~ jDate	Estimate	Std.Error
Intercept	6.623	± 0.864
jDate	-0.05	± 0.007

Vaccinium:

Table C10. Parameter estimates for the best model when modelling the proportion of Vaccinium in the diet as a function of season (Julian date, NDVI or NDSI) and year. Results from generalized linear models (GLMs) with continuous response and identity link function assuming Gaussian error distribution

Model: Vaccinium RRA ~ jDate * Year	**Estimate**	**Std.Error**
Intercept	-7.120	± 1.610
jDate	0.051	± 0.032
Year 2	-3.205	± 1.910
Year 3	3.124	± 2.128
jDate:Year 2	0.016	± 0.015
jDate:Year 3	-0.030	± 0.017

Empetrum:

Table C11. Parameter estimates for the best models when modelling the proportion of Empetrum in the diet as a function of season (Julian date, NDVI or NDSI) and year.

Model: Empetrum RRA ~ jDate	**Estimate**	**Std.Error**
Intercept	-0.904	± 0.635
jDate	-0.012	± 0.005
Model: Empetrum RRA ~ NDSI	**Estimate**	**Std.Error**
Intercept	-2.811	± 0.229
NDSI	0.703	± 0.353