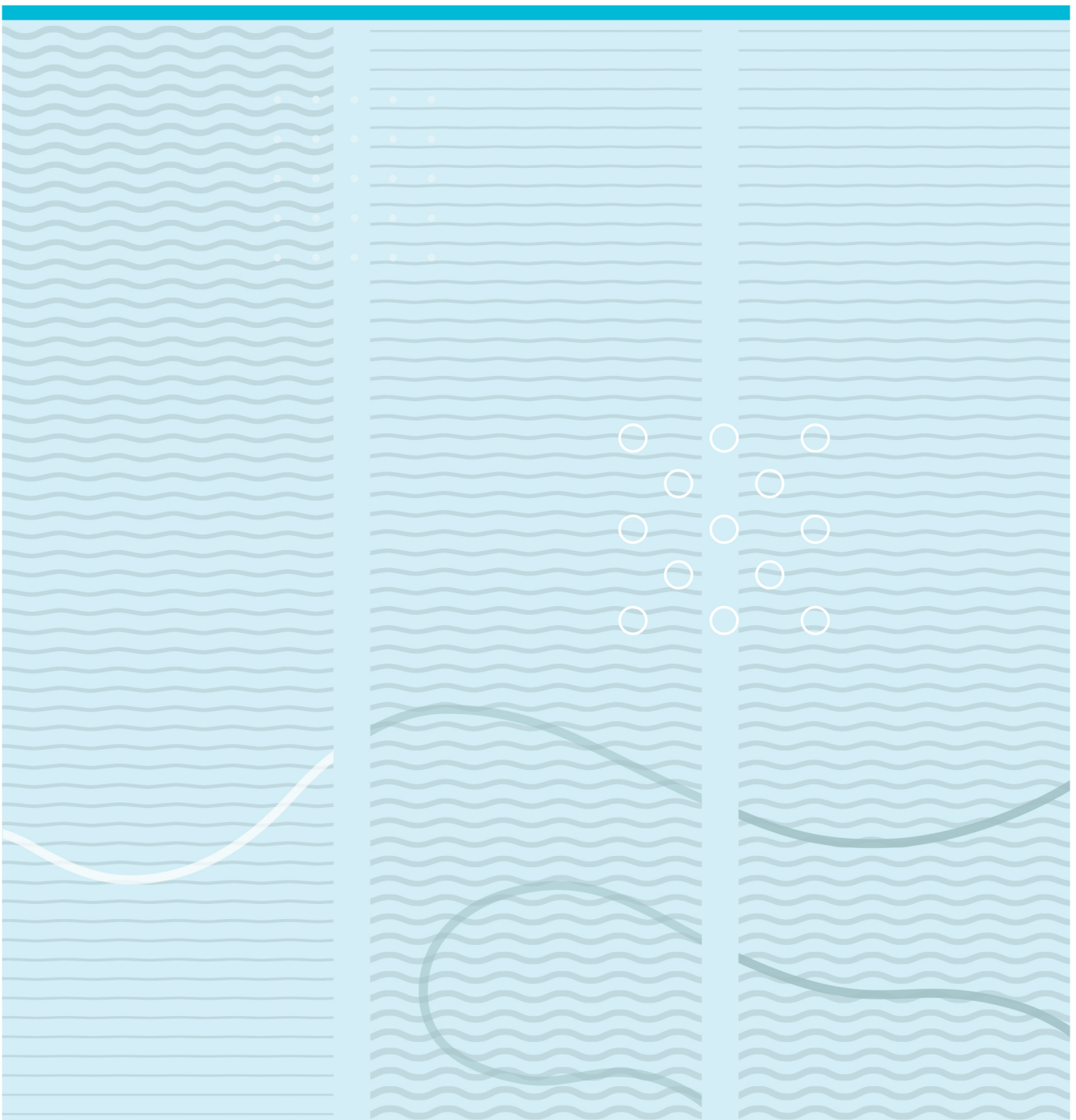


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# **Bilberry as an important resource for bumblebees within the forest-tundra ecotone**



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This thesis is worth 60 study points

## Abstract

Bumblebees are important pollinators for many plant species in high altitude environments. However, bumblebee populations currently show trends of decline as a result of several interacting drivers. Among these drivers, climate change is considered important. Global warming can cause rapid environmental changes particularly at high altitudes and has led to upslope shifts of low-altitude bumblebee species. The forest-tundra ecotone is constituted by heterogenous environments, which contain potential habitats for both low-altitude bumblebee species and bumblebee species adapted to high-altitude conditions. In spring, when floral richness is low within the ecotone, *Vaccinium myrtillus* offers large floral resources for early-emerging bumblebees. Over the course of the *V. myrtillus* flowering season, I examined how bumblebee abundance and species richness within the forest-tundra ecotone were affected by climatic factors and floral resource abundance in *V. myrtillus* communities. I additionally implemented DNA metabarcoding analysis of corbicular pollen loads from foraging bumblebees. Results from the barcoding analyses, together with observed flower visits, were then used to reveal species-specific floral preferences and temporal changes in the structure of plant-bumblebee interactions. I found that pollen compositions of the collected pollen loads largely reflected observed flower visits, suggesting that *V. myrtillus* communities provided valuable resources. I additionally found that bumblebee abundance and species richness was positively affected by local temperatures. Higher abundances of foraging bumblebees were recorded at intermediate altitude in the low alpine zone. The results also indicate that plant-bumblebee interactions were mutualistic, given the high dominance of bumblebees as visitors to *V. myrtillus*. While bumblebee pollen diets consisted of mainly *V. myrtillus* in spring, they were supplemented by other floral resources. Pollen diets in summer consisted of mainly *M. pratense*. Plant-bumblebee interactions formed generalized network structures, but the degree of specialization increased with seasonality, indicating that bumblebees narrowed their niche breadth as more flowers became available. In light of declining bumblebee populations in a changing climate, this study provide further evidence that bumblebees find valuable resources in *V. myrtillus* communities. The mutualistic plant-bumblebee interactions may further accelerate the upslope shifts of both *V. myrtillus* communities and generalist bumblebee species.

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## **Foreword**

This thesis has been carried out at the Unniversity of South-Eastern Norway (USN) with the supervision of Associate Professor Stefanie Reinhardt, Dr. Siri Lie Olsen, and Dr. Jens Åström. Part of the material for this study was collected during fieldwork in collaboration with the “Climate ecotones project” financed by the Norwegian Institute of Nature Research.

I wish to thank my supervisors for their excellent supervision, help and guidance throughout this period. Also, I would like to thank Sondre Dahle for his much appreciated help with fieldwork and lab work, Hege Brandsegg and Frode Fossøy for implementing DNA metabarcoding analyses.

# 1 Introduction

Pollinating insects are essential in both natural ecosystems (Costanza et al. 1997, Dodd et al. 1999, Montoya et al. 2012) and agricultural production (Klein et al. 2007, Gallai et al. 2009, Kleijn et al. 2015). More than three-quarters of all plant species in temperate plant communities depend on animal-pollination (Ollerton et al. 2011), where bumblebees (Hymenoptera, Apidae, *Bombus*) are considered the most important pollinators among wild bees (Corbet et al. 1991). Indeed, studies on pollinator communities in Norway have shown that bumblebees and flies are the dominating visitors to flowering plants (Kwak and Bergman 1996, Lázaro et al. 2008). Further, bumblebees have thermoregulatory abilities (Heinrich 2004), which imposes fewer constraints on foraging by weather and temperature than flies (Lundberg 1980, McCall and Primack 1992, Bergman et al. 1996), and thus provide pollination services in temperate and high altitude environments despite varying climatic conditions.

However, in recent years, trends of declines in bumblebee populations have been found in North America (Cameron et al. 2011, Jacobson et al. 2018), western and central Europe (Fitzpatrick et al. 2007, Kosior et al. 2007, Kosior et al. 2008), and Scandinavia (Dupont et al. 2011, Bommarco et al. 2012). The most potential drivers are land-use change causing loss and fragmentation of habitats, increasing use of pesticides and environmental pollution, reduced resource availability, and climate change (Potts et al. 2010, Sánchez-Bayo and Wyckhuys 2019). Under the circumstances of a warmer climate, findings show that low-altitude species shift towards higher altitudes (Kerr et al. 2015), increasing species richness where high-altitude species persevere (Miller-Struttman et al. 2015, Fourcade et al. 2019). High altitude environments are at the same time responding rapidly to climate change (Seddon et al. 2016), and cumulative evidence shows upslope expansions of shrub species and tree lines, (Harsch et al. 2009, Myers-Smith et al. 2011), leading to alterations in vegetation structure and plant species compositions (Vanneste et al. 2017, Boscutti et al. 2018). As a consequence, bumble species adapted to environmental conditions at high altitudes could be faced with competition from ascending species, experience loss of habitat area (Elsen and Tingley 2015, Rasmont et al. 2015) or loss of favoured floral resources (Miller-Struttman et al. 2015).

Bumblebees depend entirely on floral resources for survival and growth. Flower density and resource quality affect floral preferences (Fowler et al. 2016, Ruedenauer et al. 2016, Moerman et al. 2017), leading to enhanced flower constancy, ultimately increasing the fitness of favoured plant species

(Waser 1986, Brosi and Briggs 2013). However, most bumblebee species are less flower constant as they forage based on individually obtained information (Leadbeater and Florent 2014, Ruedenauer et al. 2016). Bumblebees therefore occasionally sample other available flowers during foraging trips (termed “minoring”) (Heinrich 1979). Further, despite fixed positions of bumblebee nests, foraging is extended beyond adjacent resources due to considerable foraging ranges (Westphal et al. 2006, Osborne et al. 2008). The floral composition (i.e., high density and diversity of floral resources) and spatiotemporal heterogeneity of local habitats and surroundings have thus been found to positively affect bumblebee abundance and species richness (Ranta and Vepsäläinen 1981, Hegland and Boeke 2006, Hatfield and LeBuhn 2007, Mallinger et al. 2016, Kallioniemi et al. 2017). Accordingly, corbicular pollen loads of bumblebees are usually composed of pollen from a few plant species (Mayer et al. 2012, Moquet et al. 2015, Moquet et al. 2017b).

Central and north European plant communities typically consist of six to eleven bumblebee species (Ranta and Vepsäläinen 1981). The coinciding need for floral resources and the coexistence of multiple species have therefore been discussed (Ranta and Vepsäläinen 1981, Scriven et al. 2016), particularly with regards to differences in proboscis length (Heinrich 1976, Ranta and Lundberg 1980, Klumpers et al. 2019). Foraging ranges related to differences in body and colony sizes have additionally been proposed as important factors facilitating coexistence (Westphal et al. 2006). Ranta and Vepsäläinen (1981) further argued that spatiotemporal heterogeneity in nest distribution and floral resources could explain the coexistence among bumblebees with similar proboscis length. The successive emergence of bumblebee queens from hibernation, as well as differences in peak worker abundance throughout the season (Goodwin 1995, Inoue and Yokoyama 2006), ought additionally to reduce interspecific competition (Goulson 2003).

The Forest-tundra ecotone is constituted by different transitioning plant communities as a result of elevation-related changes in biotic and abiotic conditions (Hofgaard and Wilmann 2002, Körner 2007, Klanderud et al. 2015), which contain potential habitats for both low-land bumblebee species at the edge of their climatic range and species particularly adapted to high altitude environments (Rasmont et al. 2015). In spring when floral richness is low within the ecotone and early-emerging queens start nest founding (Teräs 1976, Lundberg and Ranta 1980), spring-flowering resources are particularly vital (Carvell et al. 2017). Among the early flowering plants, *V. myrtillus* is distributed throughout the entire Norwegian forest-tundra ecotone, dominating the field layer of forest communities while also occurring in patches among heath vegetation and at the edges of snowbeds (Gjærevoll 1949, Ritchie 1956). Most ericaceous species, including *V. myrtillus*, compels pollinators

to vibrate the flower, i.e., buzz-pollinate (Jacquemart 2003), in order to maximize pollen collection due to its drooping flowers with poricidal anthers (Harder and Barclay 1994, De Luca and Vallejo-Marín 2013). Bumblebees display buzz-pollinating behaviour (Reader 1977, Javorek et al. 2002, Moquet et al. 2017a) and have indeed been described as the main pollinators of *Vaccinium* species (Reader 1977, Jacquemart 1993, Moquet et al. 2017b).

When studying mutualistic plant-pollinator networks, observed flower visits can be utilized to describe and analyse the structure of plant-pollinator interactions (Memmott 1999). However, observed flower visits seldom reveal all interactions within a plant-pollinator network (Popic et al. 2013), leading to possible overestimations of ecological specialization (Bosch et al. 2009). To decrease the proportion of unobserved plant-pollinator links, networks can be constructed from a combination of field observations and pollen analyses (Olesen et al. 2011). Yet, morphological analysis of pollen is time consuming and it is often difficult to discriminate between pollen species within the same family (Hawkins et al. 2015). Recently, DNA metabarcoding of pollen samples collected from wild bees (Potter et al. 2019), honeybees (de Vere et al. 2017), and hoverflies (Lucas et al. 2018) have been shown to successfully identify visited plant taxa, detecting more detailed plant-pollinator networks. Still, due to the possibility of biases through interspecific variation in e.g., DNA isolation efficiency, number of marker copies, and the amount of pollen produced, caution should be exercised when quantifying plant-pollinator interactions using the number of obtained sequences (Pornon et al. 2016, Bell et al. 2017).

In this study, a combination of observation based approaches and DNA metabarcoding analysis of corbicular pollen loads are used to examine the springtime foraging activity of bumblebees within a Norwegian forest-tundra ecotone. The understanding of bumblebee communities and their interactions with the surrounding environment is essential for the conservation of species (Williams and Osborne 2009). In addition, studying bumblebee communities at different altitudes can provide insight into how ongoing climate changes might affect future bumblebee populations (Burkle and Alarcón 2011, Inouye 2019). Accordingly, biotic and abiotic factors are recorded at landscape-scale to assess how floral and climatic factors affect bumblebees linked to *V. myrtillus* communities.



## 2 Material and methods

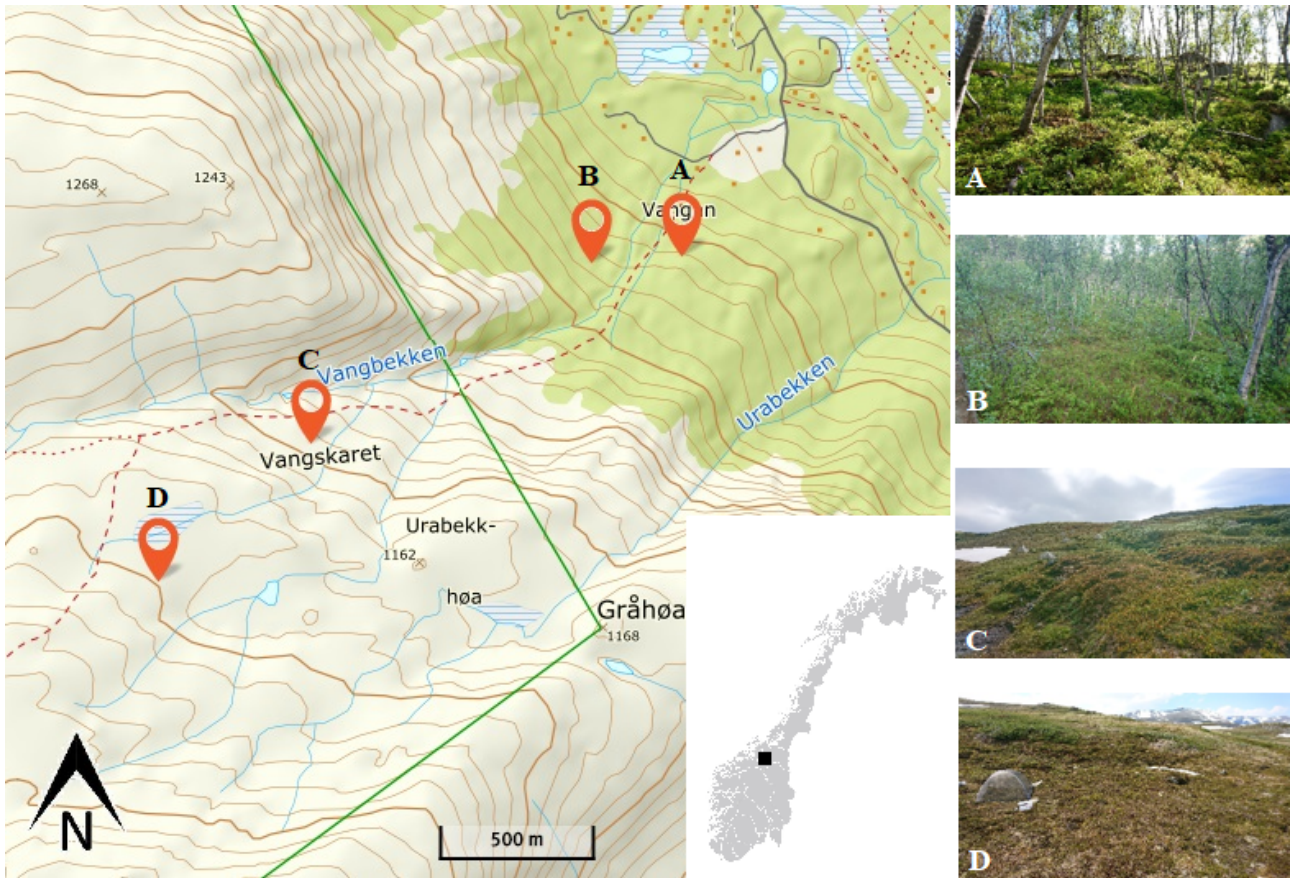
### 2.1 Study species

*V. myrtillus* is a rhizomatous dwarf shrub adapted to colder environments and poor soils (Coudun and Gégout 2007). The berries as well as vegetative parts of the plant, are of ecological importance as these resources form an essential part of the diet of e.g. rodents (Ericson 1977), brown bears (*Ursus arctos*) (Welch et al. 1997, Naves et al. 2006), ungulates (Fernández-Calvo and Obeso 2004), and is considered a major determinant in habitat selection by capercaillie (*Tetrao urogallus*) (Storch 1993). Flowering is initiated in the spring following increased temperatures and snowmelt (Selås et al. 2015) and appears more frequent at lower altitudes and in sheltered communities compared to exposed communities at higher altitudes (Ritchie 1956).

Autogamous behaviour is enabled with the aid of gravity, permitting sexual reproduction where low abundances of insects might occur (Totland et al. 2013). However, succeeding hand-pollination experiments of wild *Vaccinium* communities, seed and fruit production was reduced in *V. myrtillus* (Fröborg 1996, Guillaume and Jacquemart 1999) and *V. myrtilloides* (Reader 1977) when flowers were autogamously self-pollinated, compared to geitonogamous and xenogamous cross-pollinated flowers, emphasizing the importance of insect pollination.

### 2.2 Study area

This study is comprised of data collected in 2017 and 2018 within the forest-tundra ecotone in Grødalen (Møre og Romsdal county, midwest Norway). Landscapes in this region are characterized by a rough topography, where mountain peaks reach 1600 m.a.s.l. Mean annual precipitation at Hafsås (698 m.a.s.l., 2 km from the study site) is 675 mm. Mean monthly precipitation at Hafsås for April, May, June and July is 45 mm, 24 mm, 43 mm and, 63 mm respectively. Temperature recordings are not available from the Hafsås station, but mean annual temperature at Sunndalsøra III (10 m.a.s.l., 30 km west) is 6.7 °C. Monthly mean temperatures at Sunndalsøra III for April, May, June and July is 5.2 °C, 10.4 °C, 13.2 °C and, 14 °C respectively. All data collection was implemented at established locations, allocated to different elevations (800 – 1200 m.a.s.l.). In 2017, three locations comprised the studied locations (forest, low alpine sheltered, low alpine exposed), while an additional location (forest edge) was established in 2018 (Fig. 1, Table 1).



**Fig. 1** Map of the study site in Grødalen with the established locations; Forest at 820 m.a.s.l. (A), Forest edge at 870 m.a.s.l. (B), Low alpine sheltered at 1100 m.a.s.l. (C), Low alpine exposed at 1200 m.a.s.l. (D).

**Table 1** Established locations and plots with their specific coordinates and elevation.

Location	Plot	Longitude UTM33	Latitude UTM33	Elevation (m.a.s.l.)
Forest	1	189482.767	6948159.49	815
Forest	2	189474.227	6948158.39	816
Forest	3	189479.135	6948139.56	825
Forest	4	189470.359	6948135.34	828
Forest edge	1	189186.139	6948132.91	869
Forest edge	2	189199.322	6948144.89	862
Forest edge	3	189188.799	6948114.96	871
Forest edge	4	189185.875	6948102.57	870
Low alpine sheltered	1	188284.746	6947561.55	1093
Low alpine sheltered	2	188279.943	6947533.10	1098
Low alpine sheltered	3	188272.454	6947517.90	1102
Low alpine sheltered	4	188314.987	6947553.43	1090
Low alpine exposed	1	187737.004	6947142.99	1198
Low alpine exposed	2	187792.808	6947090.33	1204
Low alpine exposed	3	187752.488	6947009.77	1217
Low alpine exposed	4	187794.029	6947067.03	1208

### 2.3 Study design

Locations were confined to circular areas with a 50 m radius, and were chosen along a north-east facing slope, to meet the criterion of containing living and reproducing *V. myrtillus* plants. Both the forest location and the forest edge location were characterized by open forest dominated by birch (*Betula pubescens*) while *V. myrtillus* heaths dominated the understory. At both these locations, *V. myrtillus* formed a continuous cover beyond the defined border of the locations. The sheltered low alpine zone location was dominated by heaths of *Ericaceae*, dwarf birch (*B. nana*) and Willow (*Salix spp.*) At this location, *V. myrtillus* formed patches rather than a continuous cover. Due to the surrounding landscape, the location was less exposed to solar radiation and winds from south and southwest. Throughout data collection in 2018 (May – July), a patch of snow was observed covering the vegetation within the most southern part of the location. This patch of snow gradually decreased in size, allowing the covered vegetation to initiate flowering, and therefore expanded the *V. myrtillus* flowering season at the sheltered low alpine location. The exposed location in the low alpine zone was dominated by lichens and graminoids with patches of Willow and ericaceous heath vegetation. *V. myrtillus* were less abundant at this location, forming patches linked to snowbeds.

Four stationary circular plots with a 15 m radius, containing living plants of *V. myrtillus*, were chosen at all locations. Distances among plot centers was 15-50 m, depending on the distribution of *V. myrtillus* patches within locations. Researchers from the Norwegian Institute of Nature Research (NINA) collected data in 2017 on 29 June and 7 July. Sampling was on both days restricted to locations where *V. myrtillus* was flowering, which excluded sampling at the forest location on 7 July. With interest in increasing the sample size, data collection was extended throughout the *V. myrtillus* flowering season in 2018, where data were collected at all locations between 30 May and 8 July during four evenly distributed intervals (interval 1: 30 May – 3 June, interval 2: 8 June – 10 June, interval 3: 19 June – 27 June, interval 4: 4 July – 8 July). All locations were as a minimum visited three times per interval. To ensure that locations were sampled at different times during the day, the order sequence, for which locations were sampled, varied among sampling dates. In both 2017 and 2018, sampling was carried out between 09.00 and 20.00 in dry and sunny weather, enabling pollinator activity, and data collection was carried out at all locations until all *V. myrtillus* plants within the locations had terminated flowering. Data collection in 2017 and 2018 resulted in 40 and 249 periods of five minutes pollinator sampling at plots, respectively.

#### 2.4 Sampling of pollinators

Sampling of pollinators was carried out by walking at a predetermined slow pace for 5 minutes in each plot in order to detect pollinator activity. Plot observation is an advantageous method when recording associations among plants and pollinators (Westphal et al. 2008, Reitan and Nielsen 2016), facilitating valuable information for plant-pollinator webs (Memmott 1999). Pollinator visits within the plots were hence registered if a pollinator was observed in contact with a flowering plant. The species or genus of the visited plant was then recorded. Bumblebees were caught and morphologically identified to species using descriptions presented by Söderström (2013) and Ødegaard et al. (2015). Bumblebees that were not identified in the field were kept for later identification.

Workers of *B. monticola* and *B. lapponicus* are challenging to identify in the field (Gjershaug et al. 2013), and the operational taxonomic unit (OTU) *B. monticola/lapponicus* was hence used to register workers of both these species. A random selection of caught workers during sampling in 2018 was therefore kept for accurate identification. The proportion of *B. monticola* and *B. lapponicus* workers, based on the collected individuals, were then used to estimate the abundance of each species within the forest-tundra ectone. In addition, species of the subgenus *B. sensu stricto*, known to occur in Norway; *B. lucorum*, *B. terrestris*, *B. sporadicus*, *B. magnus* and, *B. cryptarum*, are difficult to distinguish when solely using morphological characteristics (Bertsch 2009). No attempt was therefore made to identify bumblebees of this subgenus and were hence registered under the OTU *B. sensu stricto*. All collected bumblebee specimens have been archived at NINA.

#### 2.5 Floral and environmental data sampling

In order to quantify the diversity and abundance of floral resources, flowering plant cover percentages of entomophilous plant taxa (hereafter referred to as flower cover) were estimated within the established circle of 50 m radius at each location. Flower cover were estimated when data collection was initiated and was continuously estimated whenever changes in flower cover were noticeable. Before instigating sampling of pollinators, temperature, cloud cover and wind speed were registered at all locations. Local temperature was measured 20 cm above ground in the shadow using an electronic thermometer. Cloud cover percentage and wind speed were roughly estimated based on observations. Interpolated values of regional daily temperature average and precipitation sum for a 1x1 km grid, were additionally obtained from The Norwegian Meteorological Institute (<https://www.met.no>) where the nearest weather stations are Innerdalen at 405 m.a.s.l., Hafsås at 698 m.a.s.l., and Sunndalsøra III at 10 m.a.s.l. As sampling of pollinators was implemented on days with no precipitation, a variable describing precipitation prior day of sampling was coupled with sampling

dates using regional daily precipitation sums. This was to explain the variation in bumblebee abundance which could be caused by an increased resource demand subsequent to days with precipitation. The temporal variation of bumblebee abundance and species richness within the 2017 and 2018 *V. myrtillus* flowering seasons were recorded in relation to day of the year. However, taking into account that onset of *V. myrtillus* flowering is correlated with preceding temperatures (Anadon-Rosell et al. 2014, Selås et al. 2015), day of season was instead determined using the preceding regional temperature averages. Approximate flowering onsets (i.e., first day of the season) were thus established subsequent to the ten first days of the year with regional daily temperature averages  $\geq 10^{\circ}\text{C}$ .

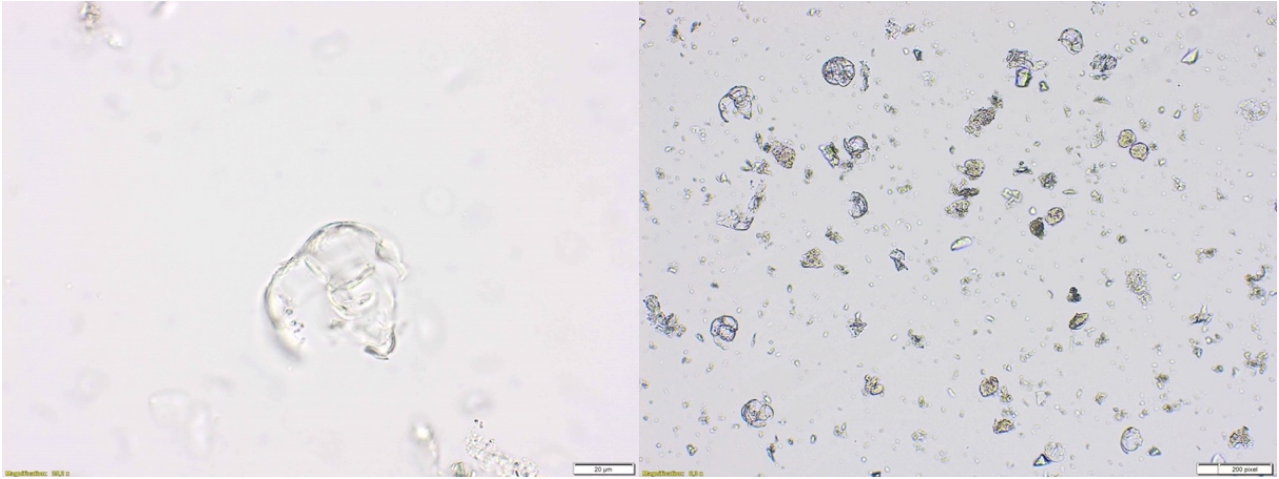
### 2.6 Collection of corbicular pollen loads

During data sampling in 2018, corbicular pollen loads (hereafter referred to as pollen loads) were collected from caught bumblebees. The collection of pollen loads was not restricted to plots. Hence, pollen loads were also collected from bumblebees within and among locations at all times. Bumblebees observed with pollen loads were immobilized in separate sterile containers using  $\text{CO}_2$  spray before being handled, to prevent cross-contamination between specimens. Pollen loads were then gently removed from the corbicula with sterile toothpicks and immediately stored in 1.5 ml plastic test tube vials containing ethanol. All vials with pollen samples were kept in cool and dark surroundings during data collection, and were subsequently stored in a freezer until further analysis. Time of day, date, elevation, habitat type, coordinates, and host plant (i.e., species/taxon of the visited plant before pollen loads were collected) were further registered for each specific pollen load sample.

### 2.7 Genetic analyses

Pollen load samples were transferred to 2 mL FastDNA Spin Kit for Soil Matrix-E tubes (MP Biomedicals) and mixed with 500  $\mu\text{L}$  EtOH. The pollen wall has high structural integrity, requiring a sufficient method for DNA extraction (Bell et al. 2016). Subsequent to homogenization tests, using a subset of collected pollen samples, pollen corns were examined under microscope to ensure satisfying disruption of the pollen wall. Acceptable results were obtained following homogenizing of the tubes at 6 m/s for 2x60 sec in a FastPrep-24 machine (MP Biomedicals) (Fig. 2). The homogenized tubes were then incubated over night at  $56^{\circ}\text{C}$ . The following day, 540  $\mu\text{L}$  ATL-buffer (Qiagen) was added and the tubes were homogenized again at 6 m/s for 60 sec. Then, 60  $\mu\text{L}$  Proteinase-K (Qiagen) were added and the samples were incubated over night a second time at  $56^{\circ}\text{C}$ . The next day, DNA

was isolated using a modified protocol based on the Qiagen DNeasy Blood and Tissue Kit (Spens et al. 2017) using 100  $\mu$ L AE-buffer for elution.



**Fig. 2** Disrupted pollen grains following homogenization at 6 m/s for 2x60 sec in a FastPrep-24 machine (MP Biomedicals).

DNA-metabarcoding followed a protocol based on the standard *Illumina 16S Metagenomic Sequencing Library Preparation* using the Nextera XT Kit (Illumina). A short region of the ITS-region was amplified in a first PCR using the ITS\_S2F/IT4 primer combination (Chen et al. 2010) with overhang adapters. Concentration of DNA-template was measured using a Nanodrop spectrophotometer and diluted approximately 10ng/ $\mu$ L using dH<sub>2</sub>O. The PCR was conducted in 25  $\mu$ L reactions with 2.5  $\mu$ L diluted template-DNA, 12.5  $\mu$ L 2x KAPA HiFi HotStart ReadyMix and 5  $\mu$ L of each primer (1 $\mu$ M). The PCR consisted of an initial 3 min step of 94°C, 40 cycles of 30 sec at 94°C, 30 sec at 56°C and 30 sec at 72 °C followed by a final phase of 10 min at 72°C. A subsequent limited-cycle amplification step was performed to add multiplexing indices. The second PCR was conducted in 50  $\mu$ L reactions with 5  $\mu$ L template-DNA, 25  $\mu$ L 2x KAPA HiFi HotStart ReadyMix and 5  $\mu$ L of each index primer. The PCR consisted of an initial 3 min step of 94°C, 8 cycles of 30 sec at 94°C, 30 sec at 55°C and 30 sec at 72°C followed by a final phase of 5 min at 72°C. PCR-products were cleaned and normalized by adding 20  $\mu$ L to a SequelPrep Normalization Plate Kit (Invitrogen) after each PCR. Finally, 10  $\mu$ L from each sample were pooled for Illumina MiSeq sequencing at the Genomics Core Facility (GCF) at the Norwegian University of Science and Technology (NTNU) in Trondheim.

## 2.8 Bioinformatic analyses

Demultiplexing were performed at GCF, and further filtering and processing of the sequences were conducted using the OBITOOLS bioinformatic package (Boyer et al. 2016) installed on a local Linux server at NINA. Briefly, forward and reverse reads were assembled using a minimum score of 20, joined sequences were removed and the dataset was dereplicated into unique reads. Finally, only reads longer than 100 bp and with at least 10 copies were kept for further analyses. Each sequence was then assigned to a taxon using a local database of all plants downloaded from the European Nucleotide Archive (ENA) ([www.ebi.ac.uk/ena](http://www.ebi.ac.uk/ena)) following OBITOOLS protocols.

The annotated sequences were next clustered at 97% identity using *sumacust* ([git.metabarcoding.org/obitools/sumacust](http://git.metabarcoding.org/obitools/sumacust)). A phylogenetic neighbour-joining tree based on the cluster centres was drawn using *Geneious* (Kearse et al. 2012). This tree was assessed by a botanist with expert knowledge of the local plant community for providing further information on taxon identity. In this way, reads with low identity could also be assigned to the correct local taxon with high confidence.

## 2.9 Statistical analysis

First, prior to any statistical analysis, data exploration was carried out following the protocol as described in Zuur et al. (2010), checking for outliers and frequencies of zeroes in the data. Next, to visualize the effect of flower cover and climatic conditions on the bumblebee species community, an ordination was generated based on data collected in 2018. Observations of bumblebee species at plots were aggregated within locations to minimize the number of zero bumblebee observations. Therefore, the ordination was based on a data matrix with columns representing the observed bumblebee species and rows representing the specific locations at specific days of the season. Due to the high proportion of zeroes, the ordination was generated using non-metric multidimensional scaling (NMDS) applied with a Bray-Curtis distance measure (Clarke 1993, McCune and Grace 2002). Calculations were computed using the R packages; *vegan* (Oksanen et al. 2018) and *goeveg* (Goral and Schellenberg 2018). In order to determine the appropriate number of ordination dimensions, the *dimcheckMDS* function was used to construct an initial run. This function provides a plot of ordination stress values for a given number of ordination dimensions, showing the decrease in stress with an increase in the number of dimensions. The initial run was made using Wisconsin double standardization, six dimensions, Bray-Curtis distance, and 1000 random iterations. Based on the stress plot, the appropriate number of dimensions were chosen where an increase in dimensions resulted in a small reduction of stress (McCune and Grace 2002). A final ordination, containing three dimensions, was generated using the *metaMDS* function. Next, a second and similarly organized data matrix

containing environmental variables (local temperature, cloud cover, wind speed, elevation, time of day, day of season, flower cover of plant taxa involved in  $\geq 5\%$  of the observed plant-bumblebee interactions: *V. myrtillus*, *V. uliginosum*, *Geranium sylvaticum*, *Melampyrum pratense*, *Phyllodoce caerulea*, *Pedicularis* spp., *Astragalus alpinus*, *Scorzoneroides autumnalis*) was added to the ordination. Permutation tests were not run as the ordination was constructed in order to visualize the dimensionality of the data set. The final ordination with the visualized explanatory variables was then further used to reveal collinearity among the explanatory variables.

Effects of floral resources and climatic factors on bumblebee abundance and bumblebee species richness were estimated using separate generalized linear mixed models (GLMM). Data from 2017 and 2018 were pooled, resulting in 289 non-independent observations as the locations were sampled multiple times. Plots nested within locations was therefore used as a random intercept to model a dependency structure (Bolker et al. 2009, Zuur et al. 2009). Year of data collection was included as a categorical fixed effect as this variable comprised two levels. Prior to modelling, all continuous explanatory variables were standardized to improve model fit, by subtracting the mean and dividing by the standard deviation. Response variables were bumblebee abundance within plots for the abundance model, and bumblebee species richness within plots for the species richness model. In both models, fixed effects of interest were fitted to assess the effects of temporal variation (day of season, time of day), climatic conditions (local temperature, regional daily temperature average, regional daily precipitation sum prior day of sampling, cloud cover, wind speed) and flower cover (cover percentages of flowering entomophilous plant species). Flower cover of *M. pratense* was excluded from the model due to a high correlation with flower cover of *G. sylvaticum*. The effect of the flower cover of *G. sylvaticum* is therefore expected to be driven by the flower cover of both *G. sylvaticum* and *M. pratense*. Further, to assess the effects of habitat types, a variable (habitat type) was included, discriminating among bumblebee observations within forest locations, the low alpine sheltered location, and the low alpine exposed location. Quadratic terms of the temporal variables (day of season, time of day) and temperature variables (local temperature and regional daily temperature average) were additionally included in the final model when these improved model fit. All continuous explanatory variables included in the model had variance inflation factor (VIF) values  $< 5$ .

Given that both response variables are count variables with excessive numbers of zero counts, appropriate distributions were applied to handle over- and underdispersion and zero-inflation, using



AIC values to determine best fit. A negative binomial distribution (i.e., the “nbinom1” distribution) with a log link function was used for the abundance model, and a Conway-Maxwell-Poisson distribution with a log link function was used for the species richness model. The *glmmTMB* function (Brooks et al. 2017) was used for both models. Model assumptions were verified by plotting model residuals versus fitted values using the R package; *DHARMA* (Hartig 2019). All statistical analyses was conducted in R (R Core Team 2018).

Plant taxa, visited more frequently than expected, were identified using resource selection null models that were based on the flower cover abundance of distinctive plant taxa. These were constructed from plant-bumblebee interactions using the R package; *econullnetr* (Vaughan et al. 2018). The null model assumes that pollinator visits are more frequent to abundant plant taxa, relative to the potential resources, as more abundant plant taxa are encountered more often. Common floral resources are therefore expected to be utilized more than rare floral resources. Following the approach of (Vaughan et al. 2018), networks were constructed using the estimated flower cover coupled with the recorded plant-bumblebee interactions. Rather than constructing a cumulative network of plant-pollinator interactions throughout the entire period of data collection, a temporal division of the data was applied (Blüthgen et al. 2006, McMeans et al. 2015), as the flowering peak of different plant species are temporally distributed. Plant-bumblebee interactions were therefore divided into “spring” and “summer”, consisting of interactions within sampling interval one and two (30 may – 10 june) and sampling interval three and four (19 june – 8 july), respectively. A spring and a summer network was thus constructed from both the observed plant-bumblebee interactions (flower visitation networks) and plant-bumblebee interactions obtained from the DNA metabarcoding analysis of pollen loads (pollen transport networks). DNA sequences were converted into proportions as suggested by de Vere et al. (2017), so as to obtain the relative amount of plant pollen taxa in each pollen load, providing a semi-quantitative approach. Sequence proportions < 1 % were further excluded from the network analysis. Also, pollen loads were collected outside established locations. Thus, flower cover of plant taxa outside established locations were assumed to resemble flower cover values at locations with similar plant species composition and vegetation structure. If a plant taxon was detected in a pollen load sample, and was not recorded at the sampled location, flower cover of that specific plant taxon was registered as 1 %. Further, flower cover of *Pedicularis* spp. was measured based on the abundance of both *P. lapponica* and *P. oederi*. The obtained plant pollen DNA sequences for these species were therefore pooled and named *Pedicularis* spp. in order to couple plant pollen DNA sequences with flower cover abundances. The four network null models were constructed using 9999 iterations, and 95 % confidence intervals were estimated. Observed network values not included in

the 95 % confidence intervals can be considered significantly higher or lower than expected under the null model assumptions. However, low numbers of recorded plant-bumblebee interactions for rarely observed bumblebee species, did not produce the sufficient amount of information required to calculate credible expected values from null models. Consequently, significantly stronger or weaker floral preferences in flower visitation networks were deemed credible for bumblebee species observed 10 times or more within each period (spring and summer), as recommended by (Vaughan et al. 2018).

The network metric  $H_2'$  was calculated from the constructed networks to describe their interaction structures (Blüthgen et al. 2006).  $H_2'$  measures the degree of generalization or specialization within an interaction network and ranges from 0 (perfect generalization) to 1 (perfect specialization). Estimations of  $H_2'$  are sufficient even for small plant-pollinator networks deprived of sampling bias, making estimations robust against sampling intensity, network shape and size (Blüthgen et al. 2006).

Having found specific plant pollen taxa preferences among different bumblebee species through network analysis, differences in pollen load compositions among bumblebee species and among host plant species were tested with permutational analysis of variance (perMANOVA). Similar to network analyses, pollen load compositions were divided into spring and summer compositions. As terms are added sequentially to the perMANOVA formula, both possible order sequences were tested. The test was executed using the *adonis* function in the R package; *vegan* (Oksanen et al. 2018), using the Jaccard similarity index and 9999 permutations. As the Jaccard similarity index uses presence-absence data, the presence of rare plant taxa might be overemphasized. Plant pollen taxa contributing to less than 1 % of the relative proportion of DNA sequences in each pollen sample were therefore excluded. Due to an independent study design where locations were sampled multiple times, the *strata* argument in *adonis* was used to apply a dependency structure, in the same way random effects are applied in generalized linear mixed models. Further, bumblebee species and host plant species, for which one pollen load sample was obtained, were excluded from analysis. Pairwise comparisons of the pollen load compositions among bumblebee species and host plant species were implemented using the *adonis.pair* function in the R package; *EcolUtils* (Salazar 2019), using Bonferroni corrections to correct for multiple comparisons. Prior to analysis, homogeneity of within-group dispersions were verified using the *betadisper* function in the R package; *vegan* (Oksanen et al. 2018).

## 3 Results

### 3.1 Overview

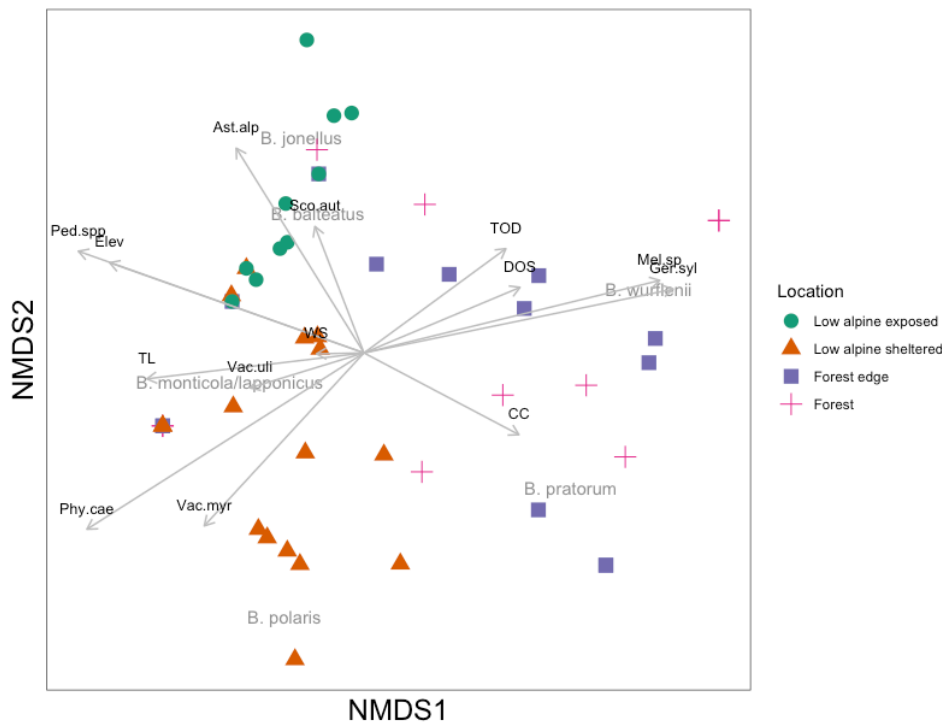
In total, 355 individuals of foraging bumblebees were observed during the two years of this study, comprising ten different bumblebee OTU's (listed in order of descending abundance); *B. monticola/lapponicus* (where 34/50 collected individuals were identified as *B. monticola*, and 16/50 were identified as *B. lapponicus*), *B. pratorum*, *B. wurflenii*, *B. balteatus*, *B. jonellus*, *B. polaris*, *B. sensu stricto*, *B. alpinus*, *B. pascuorum* and, *B. consobrinus*. Observations were dominated by *B. monticola/lapponicus* (65 %) (Appendix 1).

A total of 277 plant-bumblebee interactions were observed throughout data collection in 2018, involving 14 different plant taxa, where eight plant taxa represented more than 90 % of all observed visits (listed in descending order); *G. sylvaticum*, *V. myrtillus*, *V. uliginosum*, *M. pratense*, *P. caerulea*, *Pedicularis* spp., *A. alpinus*, *S. autumnalis* (for a complete list see Appendix 2).

The collection of corbicular pollen loads amounted to 78 samples from nine different bumblebee OTU's (listed in order of descending abundance): *B. monticola/lapponicus*, *B. sensu stricto*, *B. wurflenii*, *B. pratorum*, *B. jonellus*, *B. polaris*, *B. balteatus*, *B. pascuorum*, *B. hypnorum* (Appendix A1). Sequencing yielded 10177200 DNA sequences over 100 bp in length. From these DNA sequences, 59 different plant taxa could be identified to species, genus or family level, where six species constituted more than 90 % of all sequences (listed in order of descending abundance); *V. myrtillus*, *M. pratense*, *P. lapponica*, *P. oederi*, *A. alpinus*, *P. caerulea* (for a complete list of plant pollen taxa DNA sequences contributing to more than 1 % of corbicular pollen load samples, see Appendix 2). Overall, 35 % of pollen load samples were monospecific (i.e., > 95 % of conspecific pollen), where *M. pratense*, *V. myrtillus*, *A. alpinus*, and *P. lapponica* constituted the conspecific plant pollen species in 52 %, 30 %, 11 %, and 7 % of the monospecific pollen load samples, respectively.

### 3.2 Bumblebee species composition

The ordination biplot shows the distribution of observed bumblebee species as well as the relative importance of the measured environmental and temporal variables on the bumblebee community within the forest-tundra ecotone (Fig. 3). Axis 1 was primarily a function of differences in bumblebee species composition between the forest locations and the low alpine locations, corresponding to a forest-tundra gradient. The positions of the forest and the forest edge locations were overlapping in the NMDS ordination space indicating that similar bumblebee species compositions were recorded at these locations.



**Fig. 3** Ordination triplot of recorded bumblebee species compositions during the *V. myrtillos* flowering season in 2018 with non-metric multidimensional scaling (NMDS). Symbols represent the bumblebee species composition at established locations within the forest-tundra ecotone (forest, forest edge, low alpine sheltered, low alpine exposed) ( $n = 49$ ). Non-metric multidimensional scaling is based on Bray-Curtis similarity (stress = 0.079). Vector labels refer to environmental and temporal variables. Flowering plant cover abundance variables; Ast.alp = *Astragalus alpinus*, Ger.syl = *Geranium sylvaticum*, Mel.sp = *Melampyrum pratense.*, Ped.spp = *Pedicularis* spp., Phy.cae = *Phyllodoce caerulea*, Sco.aut = *Scorzoneroideis autumnalis*, Vac.myr = *Vaccinium myrtillos*, Vac.uli = *Vaccinium uliginosum*. Abiotic variables; TL = Local temperature, CC = Cloud cover, Elev = Elevation, WS = Wind speed. Temporal variables; DOS = Day of season, TOD = Time of day.

Axis 2 was primarily a function of differences in bumblebee species compositions between the low alpine sheltered and the low alpine exposed locations as these locations were separated in two groups in the ordination space. The three bumblebee species composition clusters (i.e., the forest locations, the low alpine sheltered location and the low alpine exposed location) were largely differentiated by

floral resource compositions. *B. pratorum* and particularly *B. wurflenii* were associated with the forest and the forest edge locations. Recorded observations of *B. wurflenii* was additionally highly correlated with high flower cover of *G. sylvaticum* and *M. pratense*. *B. monticola/lapponicus* and *B. polaris* were mostly associated with the low alpine sheltered location and high flower cover of the ericaceous species *V. myrtillus*, *V. uliginosum* and, *P. cearulea*. Recorded observations of *B. jonellus* and *B. balteatus* were associated with the highest elevated location, the low alpine exposed location, where *A. alpinus* and *S. autumnalis* was correlated with recorded bumblebee observations (Fig. 3).

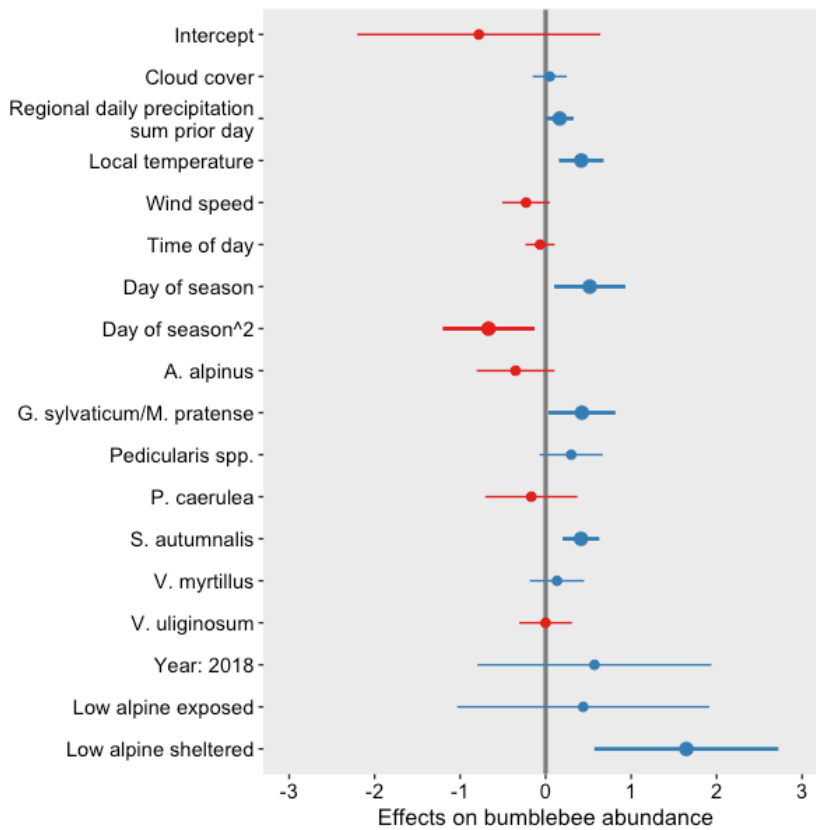
### 3.3 Bumblebee abundance and species richness

The variation in bumblebee species richness was to a great extent explained by bumblebee abundance (Appendix 4). The model estimates of the effects therefore mirrored the effects on bumblebee abundance but were generally less certain and are therefore not presented here.

Local temperature and regional daily precipitation sum prior day of sampling had positive effects on bumblebee abundance (Fig. 4, Table 2, Appendix 3A and 3B). Locations with one standard deviation higher temperatures had on average 52 % (95% conf.int: 16% to 97%) more individuals of bumblebees and locations with one standard deviation higher precipitation sum prior day of sampling had on average 17 % (95% conf.int: 0% to 39%) more individuals of bumblebees. Including local temperature as a quadratic term, allowing for a local temperature optimum, did not improve the model, indicating that foraging bumblebees were not restricted by high local temperatures within the temperature range of this study.

Wind speed had an estimated negative effect on bumblebee abundance, however the confidence interval included zero (95% conf.int: -40% to 5%), constituting an uncertainty of the effect. Cloud cover (95% conf.int: -14% to 28%) and time of day (95% conf.int: -21% to 12%) had no evident effect on bumblebee abundance given the low model estimates and confidence intervals largely overlapping zero (Fig. 4, Table 2).

Bumblebee abundance increased with day of season. We observed on average, 68 % (95% conf.int: 1% to 153%) more individuals of bumblebees at locations with an one standard deviation linear increase in day of season. Additionally, including day of season as a quadratic term improved the model (95% conf.int: -70% to -12%), indicating a bumblebee abundance optimum late in the *V. myrtillus* flowering season (Fig. 4, Table 2, Appendix 3C).



**Fig. 4** Results from the generalised linear mixed model of bumblebee abundance with negative binomial distribution and log link function. Lines represent the 95 % confidence interval and dots represent the model estimate for each effect. Blue and red colors indicate whether model estimates are positive or negative, respectively. Confidence intervals not including zero are shown with thicker dots and lines.

**Table 2** Model output of the fixed effects included in the generalised linear mixed model explaining bumblebee abundances in the forest-tundra ecotone during the *V. myrtillus* flowering season in 2017 and 2018. The different plant taxa included in the model, are estimated effects of flowering plant cover on bumblebee abundance. The habitat type variable shows the estimated effect on bumblebee abundance in the forest habitat (Intercept), the exposed low alpine habitat (Low alpine exposed), and the sheltered low alpine habitat (Low alpine sheltered).

	Estimate	Std. error	z-value	p-value
Intercept	-0.781	0.725	-1.077	0.28
Cloud cover	0.048	0.101	0.475	0.63
Regional daily precipitation sum prior day	0.164	0.083	1.987	0.05
Local temperature	0.416	0.134	3.117	0.00
Wind speed	-0.229	0.142	-1.161	0.11
Time of day	-0.065	0.087	-0.749	0.45
Day of season	0.516	0.213	2.428	0.02
Day of season^2	-0.668	0.274	-2.436	0.02
<i>A. astragalus</i>	-0.352	0.232	-1.517	0.13
<i>G. sylvaticum/M. pratense</i>	0.424	0.200	2.118	0.03
<i>Pedicularis spp.</i>	0.299	0.188	1.588	0.11
<i>P. caerulea</i>	-0.168	0.275	-0.611	0.54
<i>S. autumnalis</i>	0.413	0.110	3.772	0.00
<i>V. myrtillus</i>	0.133	0.162	0.821	0.41
<i>V. uliginosum</i>	-0.001	0.157	-0.008	0.99
Year: 2018	0.571	0.699	0.817	0.41
Low alpine sheltered	1.646	0.549	3.000	0.00
Low alpine exposed	0.439	0.752	0.584	0.56

Interestingly, most included flower cover variables comprised uncertain effects (Fig. 4, Table 2). Particularly, the effects of the ericaceous species flower cover on bumblebee abundance were uncertain with low model estimates and confidence intervals largely overlapping zero; *V. myrtillus* (95% conf.int: -16% to 57%), *V. uliginosum* (95% conf.int: -27% to 36%), and *P. caerulea* (95% conf.int: -51% to 45%). Flower cover of *Pedicularis* spp. had a positive effect on bumblebee abundance, but the confidence interval included zero (95% conf.int: -7% to 95%), constituting uncertainty of the effect. Flower cover of *G. sylvaticum/M. pratense* and *S. autumnalis* had similar positive model estimates, but the effect of *G. sylvaticum/M. pratense* was more uncertain (Fig. 4, Table 2, Appendix 3D and 3E). Locations with one standard deviation more flower cover of *G. sylvaticum/M. pratense* had on average 52 % (95% conf.int: 3% to 127%) more individuals of bumblebees and locations with one standard deviation more flower cover of *S. autumnalis* had on average 51 % (95% conf.int: 22% to 88%) more individuals of bumblebees. *S. autumnalis* and *A. alpinus* were flowering during the four last days of data collection within the low alpine exposed location, with flower cover of *S. autumnalis* being highest during the last day of data collection. The flower cover of *S. autumnalis* and *A. alpinus* were therefore moderately correlated (correlation coefficient 0.66). However, the effect of *A. alpinus* flower cover was uncertain with a confidence interval overlapping zero (95% conf.int: -46% to 11%) (Fig. 4, Table 2).

The defined habitat types within the forest-tundra ecotone were included in the model to test whether unmeasured factors, related to the different habitat types, could help explain the variation of bumblebee abundances. While the model results revealed great uncertainty of the effects of forest habitats and the low alpine exposed habitat, they clearly indicate that bumblebee abundances were higher within the low alpine sheltered habitat type throughout the *V. myrtillus* flowering season (Fig. 4, Table 2).

Plots nested within locations as a random intercept accounted for 17 % of the variation with an estimated variance of 0.24.

### 3.4 Floral preferences

Floral preferences of three different bumblebee species were found in the flower visitation networks and of seven different bumblebee species in the pollen transport networks (Fig. 5, Table 3, Appendix 5, Appendix 6). The spring flower visitation network showed a strong preference for *V. myrtillus* by *B. monticola/lapponicus*, and *V. myrtillus* pollen was collected more than expected, compared to the null model, by *B. monticola/lapponicus*, *B. jonellus* and, *B. pratorum* in spring.

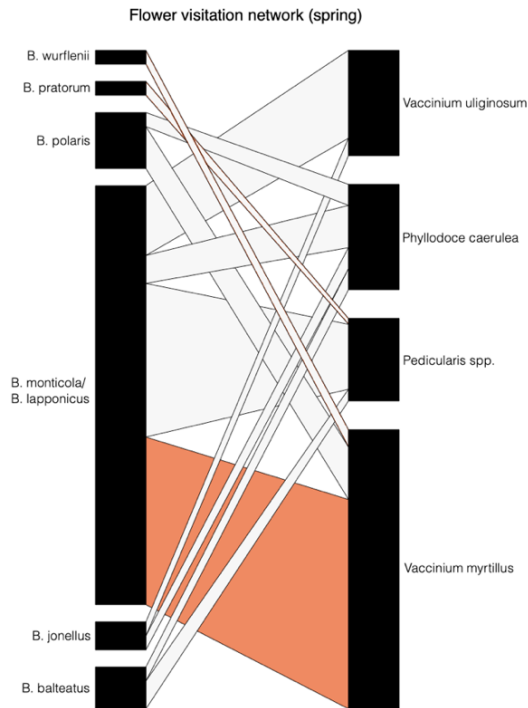
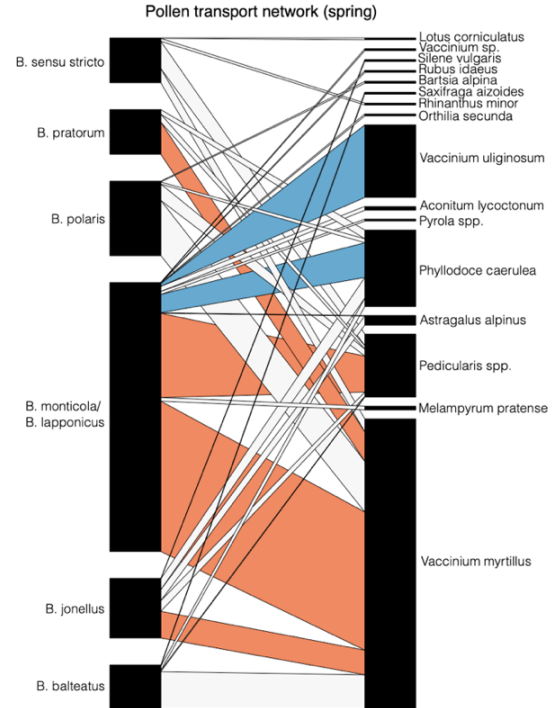
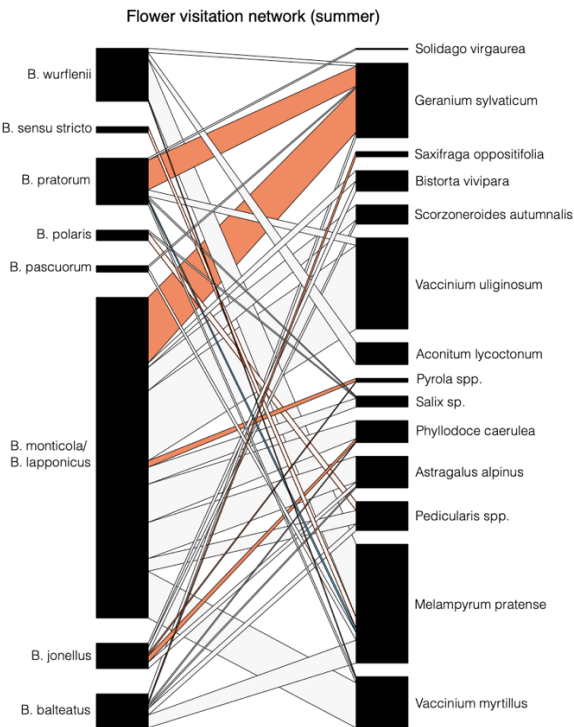
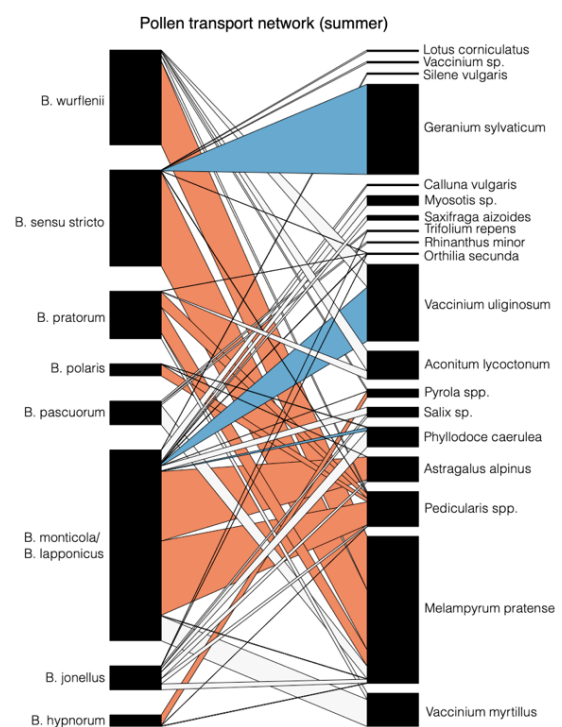
Pollen transport interactions in summer showed that pollen of *M. pratense* was collected more than expected, compared to the null model, by *B. wurflenii*, *B. sensu stricto* and, *B. pratorum*. Pollen of *Pedicularis* spp. was collected more than expected by *B. polaris*, *B. pratorum* and, *B. monticola/lapponicus*. In addition, pollen of *A. alpinus* was collected more than expected, compared to null models, by *B. monticola/lapponicus*. The summer flower visitation network revealed that *G. sylvaticum* was visited more than expected, compared to the null model, by *B. monticola/lapponicus* and *B. pratorum*, and *Pyrola* spp. was visited more than expected, compared to the null model, by *B. monticola/lapponicus*. Further, *P. caerulea* was visited more than expected, compared to the null model, by *B. jonellus*.

Weak floral preferences were additionally revealed. Pollen from *V. uliginosum* and *P. caerulea* was collected less than expected, compared to the null model, by *B. monticola/lapponicus* in spring and summer, while pollen from *G. sylvaticum* was collected less than expected, compared to the null model, by *B. sensu stricto* in summer (Fig. 5, Table 3, Appendix 6). Indeed, pollen of *V. uliginosum* and *G. sylvaticum* was overall collected surprisingly little (both these species contributed to less than 0.5 % of all plant pollen DNA sequences), given their involvement in more than 30 % of observed plant-bumblebee interactions (Appendix 2). Similarly, *B. pratorum* was observed on *M. pratense* less than expected, given their strong preference for *M. pratense* pollen, as indicated by the pollen transport network.



**Table 3** Plant taxa with significantly more or less visits than expected from null models during spring and summer based on plant-bumblebee interactions recorded through observations and DNA metabarcoding analysis of corbicular pollen loads. Values of observed visits (flower visitation networks) and summed proportions of corbicular pollen loads (pollen transport networks) are shown with lower and upper bounds of 95 % confidence intervals from the null models in brackets. <sup>a</sup>Values indicate more visits and <sup>b</sup>values indicate less visits than expected under the null model.

Plant species/taxon	Bumblebee species	Flower visitation network	Pollen transport network
<b>Spring</b>			
<i>Pedicularis</i> spp.	<i>B. monticola/lapponicus</i>	-	5.59 (0.82 - 4.71) <sup>a</sup>
<i>Phyllodoce caerulea</i>	<i>B. monticola/lapponicus</i>	-	1.21 (1.27 - 5.74) <sup>b</sup>
	<i>B. jonellus</i>	-	1.77 (0.00 - 0.84) <sup>a</sup>
<i>Vaccinium myrtillus</i>	<i>B. monticola/lapponicus</i>	12 (3 - 9) <sup>a</sup>	9.96 (2.63 - 5.99) <sup>a</sup>
	<i>B. pratorum</i>	-	2.13 (0.25 - 1.66) <sup>a</sup>
<i>Vaccinium uliginosum</i>	<i>B. monticola/lapponicus</i>	-	0.17 (3.38 - 7.95) <sup>b</sup>
<b>Summer</b>			
<i>Astragalus alpinus</i>	<i>B. monticola/lapponicus</i>	-	5.75 (1.21 - 4.92) <sup>a</sup>
	<i>B. pratorum</i>	14 (0 - 14) <sup>a</sup>	-
<i>Geranium sylvaticum</i>	<i>B. monticola/lapponicus</i>	31 (1 - 30) <sup>a</sup>	-
	<i>B. sensu stricto</i>	-	0.02 (0.73 - 4.23) <sup>b</sup>
	<i>B. pratorum</i>	1 (2 - 16) <sup>b</sup>	2.13 (0.04 - 1.87) <sup>a</sup>
<i>Melampyrum pratense</i>	<i>B. sensu stricto</i>	-	7.79 (1.92 - 5.71) <sup>a</sup>
	<i>B. wurflenii</i>	-	6.92 (1.72 - 5.45) <sup>a</sup>
	<i>B. monticola/lapponicus</i>	-	6.14 (1.05 - 5.06) <sup>a</sup>
<i>Pedicularis</i> spp.	<i>B. pratorum</i>	-	1.27 (0.00 - 0.96) <sup>a</sup>
	<i>B. polaris</i>	-	0.96 (0.00 - 0.82) <sup>a</sup>
	<i>B. jonellus</i>	3 (1 - 2) <sup>a</sup>	-
<i>Phyllodoce caerulea</i>	<i>B. monticola/lapponicus</i>	-	0.05 (0.12 - 2.98) <sup>b</sup>
	<i>B. hypnorum</i>	-	0.89 (0.00 - 0.25) <sup>a</sup>
<i>Pyrola</i> spp.	<i>B. jonellus</i>	1 (0 - 1) <sup>a</sup>	-
	<i>B. monticola/lapponicus</i>	3 (0 - 3) <sup>a</sup>	-
<i>Vaccinium uliginosum</i>	<i>B. monticola/lapponicus</i>	-	0.17 (2.08 - 6.57) <sup>b</sup>

**A****B****C****D**

**Fig. 5** Flower visitation networks constructed from observed plant-bumblebee interactions during spring (A) and summer (C), and plant-bumblebee interactions obtained from corbicular pollen analysis results during spring (B) and summer (D). Bars on the left show the different bumblebee species and bars on the right show the different plant taxa. Bar lengths indicate the relative abundance of the different bumblebee species and plant taxa. Link widths represent the frequency of interactions. Orange links indicate a stronger floral preference than expected from the null model, and blue links indicate a weaker floral preference than expected

### 3.5 Network structure

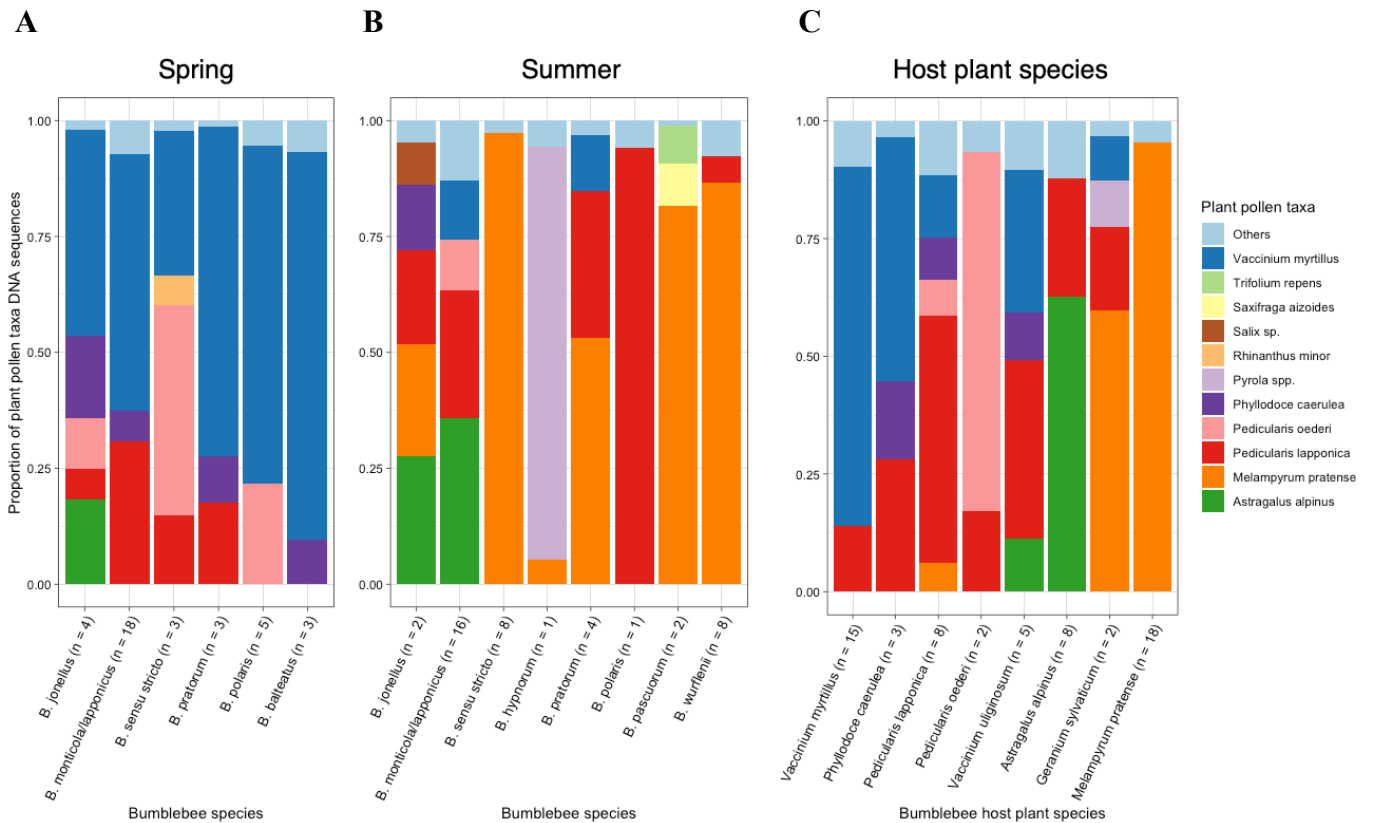
$H_2'$  measures the degree of generalization or specialization within an interaction network and ranges from 0 (perfect generalization) to 1 (perfect specialization). Values of  $H_2'$  were lower in spring compared to summer networks. The degree of network specialization in the spring flower visitation network was as expected under the null model; 0.23 (95% conf.int: 0.19 to 0.48), while the spring pollen transport network was more generalized than expected under the null model; 0.15 (95% conf.int: 0.21 to 0.38) (Table 4). These observed low degrees of network specialization indicate that the bumblebees visited multiple plant species, and the plant species were visited by multiple bumblebee species. The degree of network specialization was higher than expected, under the null model, in both the summer flower visitation network; 0.43 (95% conf.int: 0.24 to 0.39) and the summer pollen transport network; 0.52 (95% conf.int: 0.33 to 0.47), indicating that both plants and bumblebees interacted with fewer different species (Table 4).

**Table 4**  $H_2'$  values for the constructed plant-bumblebee networks. The values have been calculated from the flower visitation networks and the pollen transport networks during spring and summer (Fig. 5). <sup>a</sup>Values indicate higher values and <sup>b</sup>values indicate lower values than expected under the null model.

Network metric	Spring networks		Summer networks	
	Flower visitation	Pollen transport	Flower visitation	Pollen transport
$H_2'$	0.23	0.16 <sup>b</sup>	0.43 <sup>a</sup>	0.52 <sup>a</sup>

### 3.6 Corbicular pollen load compositions

Pollen of *V. myrtillus* dominated pollen load compositions in spring, while pollen of *M. pratense* dominated pollen load compositions in summer (Fig. 6A and 6B). Overall, the diversity of plant pollen compositions in pollen loads appear to decrease with increasing proboscis length, although there are some exceptions (Fig. 6A and 6B). Bumblebees with ericaceous host plant species appear to collect relatively similar pollen species, while bumblebees with herbaceous host plant species appear to differentiate in pollen composition. Pollen of *P. lapponica* appears to be collected regardless of host plant species, with the exception of *M. pratense* (Fig. 6C).



**Fig. 6** Mean proportions of plant pollen taxa DNA sequences obtained from bumblebees within the forest-tundra ecotone during the *V. myrtillus* flowering. DNA sequence proportions are illustrated for spring (A) and summer (B), and for the most frequently visited host plant species (C). Bumblebee species, along the x-axis, are listed in order of increasing proboscis length, obtained from Ranta (1982). Host plant species are listed in order of observed flowering peak. n denotes the number of samples for each bumblebee species and host plant species. For simplicity, plant taxa contributing less than 5 % of the different bumblebee species and host plant species pollen compositions, are categorized as “Others”.

PerMANOVA tests of pollen compositions in spring showed a clear difference in plant pollen taxa composition among bumblebee host plant species ( $F_{(3,22)} = 2.24$ ,  $R^2 = 0.26$ ,  $p = 0.040$ ) when host plant species was included as the first term in the perMANOVA formula (Fig. 6C). There was however no clear difference in plant pollen taxa composition among bumblebee species in spring (Fig. 6A).

PerMANOVA tests of pollen compositions in summer showed a clear difference in plant pollen taxa composition among bumblebee host plant species ( $F_{(4,32)} = 13.35$ ,  $R^2 = 0.66$ ,  $p = 0.030$ ) and among bumblebee species ( $F_{(5,32)} = 9.15$ ,  $R^2 = 0.56$ ,  $p = 0.038$ ) when terms term were included as the first term in the perMANOVA formula (Fig. 6B and 6C, Appendix 8).

Post hoc pairwise comparisons among summer foraging bumblebee species revealed clear differences in plant pollen taxa compositions of pollen loads between *B. wurflenii* - *B. monticola/lapponicus* (Bonferroni  $p = 0.002$ ), and *B. monticola/lapponicus* - *B. sensu stricto* (Bonferroni  $p = 0.002$ ) (Fig. 6A and 6B).

Post hoc pairwise comparisons among spring host plant species revealed that there was a clear difference in plant pollen taxa compositions of pollen loads between *V. myrtillus* - *P. lapponica* (Bonferroni  $p = 0.021$ ). Post hoc pairwise comparisons among summer host plant species revealed that there was a clear difference in plant pollen taxa compositions of pollen loads between *M. pratense* - *A. alpinus* (Bonferroni  $p = 0.001$ ), *M. pratense* - *P. lapponica* (Bonferroni  $p = 0.013$ ) (Fig. 6C).

## 4 Discussion

In this study, I combined observations and DNA metabarcoding analysis of pollen loads to examine floral and climatic effects on bumblebees linked to *V. myrtillus* communities within the forest-tundra ecotone.

Bumblebee abundance and species richness was affected by both climatic and floral factors. Local temperatures and precipitation prior day of sampling had positive effects on the foraging bumblebees, while wind speed appeared to have a negative effect. Flower cover of *G. sylvaticum*, *M. pratense*, and *S. autumnalis* positively affected bumblebee occurrences.

The majority of pollen loads belonged to plant taxa present in *V. myrtillus* communities, with particularly high abundances of *V. myrtillus*, *M. pratense* and *Pedicularis* spp. In addition, pollen loads largely reflected the observed flower visit interactions, indicating that *V. myrtillus* communities offer valuable floral resources for spring foraging bumblebees within the forest-tundra ecotone. These results support previous studies on bumblebee communities in heathlands (Mayer et al. 2012, Moquet et al. 2015, Moquet et al. 2017b), open subalpine fields (Kwak and Bergman 1996), and forest meadows (Jennersten and Kwak 1991).

### 4.1 *V. myrtillus* as an floral resource

*V. myrtillus* dominated the collected pollen loads in the spring and was utilized by all spring foraging bumblebee species. Previous studies suggest that bumblebees sample different plant species while foraging, in order to continuously assess the quality of floral resources (Heinrich 1979, Leadbeater and Florent 2014, Vanderplanck et al. 2014), and both high nectar quality and floral abundance have been shown to increase bumblebee visits to flowers (Fowler et al. 2016). In the spring during colony development, pollen resources are particularly vital (Ruedenauer et al. 2016). Findings from studies in Belgium heathlands indicate that *V. myrtillus* offer high amounts of sucrose rich nectar and produce pollen of poor quality in relatively high amounts (Jacquemart 2003, Moquet et al. 2015). Thus, one explanation for the common utilization of *V. myrtillus* in spring could be high amounts of nectar and pollen, which can be gathered from flowers that are available in high densities and high abundances. To compensate for a poor pollen quality, bumblebees might consume larger quantities of nectar to improve the cost-benefit balance of foraging activity (Vanderplanck et al. 2014) or mix plant pollen species (Moquet et al. 2015), which may explain the additional use of other available resources.

In addition, *V. uliginosum*, *G. sylvaticum*, and, *S. autumnalis* stood for a large portion of flower visits by bumblebees. However, pollen of these species was scarcely represented in the corbicular pollen loads, indicating that they were visited mainly for nectar. This finding is in contrast to Mayer et al. (2012), who found that *V. uliginosum* constituted the main pollen resource of bumblebees in heathlands. Pollen collection from ericaceous species (such as *V. uliginosum*) is enabled through buzz-pollination (De Luca and Vallejo-Marín 2013), which suggests that *V. uliginosum* could function as a valuable pollen source for bumblebees. In my study, other flowering ericaceous species, offering floral resources, were utilized for pollen resources in proportions roughly matching their abundances. However, lower amounts of *V. uliginosum* pollen per flower compared to flowers of *V. myrtillus* (Jacquemart 2003), might suggest that the costs of collecting pollen from *V. uliginosum* surpass the benefits for bumblebees, when other resources are available. Other possible explanations for this result includes a lower detectability of this species in the DNA metabarcoding analysis. For example, pollen grains of *V. uliginosum* might contain low copy numbers of ITS sequences or be particularly resistant against disruption of the pollen wall, hindering proper DNA extraction. This would result in an underestimation of its presence in pollen load samples. But there is no reason why *V. uliginosum* would be particularly affected, so this potential error source applies to the DNA metabarcoding results in general.

#### 4.2 Effects on bumblebee abundance and species richness

The present study shows that bumblebee abundance and bumblebee species richness was clearly higher at the sheltered low alpine location. There might be several explanations for this finding. The relationship between plant species richness and altitude is often unimodal, meaning that floral richness is higher at intermediate altitudes (Bruun et al. 2006, Hoiss et al. 2015). The high floral richness of the low alpine location might therefore offer relatively continuous supplies of floral resources for generalist bumblebees (Mallinger et al. 2016). Further, the landscape topography and vegetation structure, surrounding the sheltered low alpine location, may provide optimal nesting sites for bumblebees (Hatfield and LeBuhn 2007). Consequently, bumblebee nest density may have been higher within the habitat surrounding the sheltered low alpine location. An alternative explanation is that bumblebee colonies with large foraging ranges, located at both lower or higher elevations, might utilize floral resources at intermediate elevations whenever gaps occur in the transition of flowering phenologies.

While the positive effect of *G. sylvaticum* and *M. pratense* flower cover on bumblebee abundance and species richness was substantiated by the detected floral preferences of several bumblebee

species, effects of the ericaceous species flower cover were uncertain. These unclear effects may be explained by the wide distribution of the ericaceous species within the ecotone. While the floral composition within the ecotone was relatively homogenous in spring, it appeared that the flower cover of ericaceous species were mostly associated with foraging activities at the sheltered low alpine location, as was indicated in the NMDS ordination. In other words, the effect of ericaceous species flower cover could be less pronounced at other locations within the ecotone.

Further, bumblebee abundance and species richness was positively affected by the flower cover of *Pedicularis* spp., but the effect was uncertain. This could be caused by the low density of flower patches in which *Pedicularis* plants were present, as individuals of this plant taxa were often dispersed rather than clustered in groups. Dispersed flowers may be less attractive for bumblebees (Hegland and Boeke 2006), and the visitation rate may therefore have been higher when *Pedicularis* flowers intermingled with other plants (Kwak and Bergman 1996).

While flower cover of *S. autumnalis* had a positive effect on bumblebee abundances and species richness, the effect of *A. alpinus* was negative but the effect was uncertain. The flower cover abundance of these species were moderately correlated as they both formed densely intermingled flower patches at the exposed low alpine location. Further, a closer look at the recorded flowering phenology showed that *A. alpinus* reached peak flower cover abundance a few days in advance of *S. autumnalis*, during the second flowering period of *V. myrtillus*. Thus, floral resources of *V. myrtillus* may have attracted foraging bumblebees at the expense of *A. alpinus*. Then, as *V. myrtillus* had terminated flowering, floral resources offered by both *A. alpinus* and *S. autumnalis* may have become highly attractive.

Bumblebee abundance and species richness was positively affected by local temperature, which is in congruence with previous studies (Lundberg 1980, McCall and Primack 1992, Bergman et al. 1996, Sanderson et al. 2015). Foraging at low temperatures is enabled through thermoregulation, which is energetically costly (Heinrich 2004). Further, Corbet et al. (1988) found that buzz frequencies increased with ambient temperatures, and suggested that high ambient temperatures lead to maximal muscle efficiency. Energy requirements for thermoregulation might therefore decrease at higher temperatures, allowing bumblebees to spend more energy on flight and optimize the output of buzz-pollination. Based on the temperature measures included in this study, local temperature explained the bumblebee occurrences better than the interpolated daily averages from the meteorological institute. This finding is in contrast to Nielsen et al. (2017), where they found daily temperature



averages to be the best temperature measure, explaining the variation of bumblebee visits to raspberry fields in farmlands. One explanation for this difference could be different study environments. If climatic conditions are spatially heterogeneous at high altitudes, local temperatures might explain the variation in bumblebee abundance and species richness better. Moreover, I did not find a local temperature optimum, suggesting that the local temperatures, within the recorded range of this study, did not represent an activity barrier for bumblebees.

Bumblebee abundance and species richness increased with increasing amounts of precipitation prior day of sampling. Previous studies have shown that rain is a limiting factor for bumblebee activity (Teräs 1976, Lundberg 1980, Sanderson et al. 2015). Hence, increased foraging after periods with rain could be a behavioural response in order to replenish the food supplies of the colony. Few consecutive days with rain during the time of data sampling might explain the weak effect. Thus, prolonged episodes with rain could possibly cause profound depletions of colony food storages.

In line with previous studies (Lundberg 1980, McCall and Primack 1992, Bergman et al. 1996, Sanderson et al. 2015), wind speed had a negative, but uncertain, effect on bumblebee abundance and species richness. Lundberg (1980) proposed that bumblebees, particularly adapted to high altitude environments, were better able to forage in winds of relatively high velocity. Bergman et al. (1996) observed that bumblebees flew closer to the ground when wind speeds were high and found that bumblebee activity was limited when wind speeds exceeded an hourly mean of  $8 \text{ m s}^{-1}$ . In the present study, wind speeds were highest at the exposed low alpine location, where the landscape topography and vegetation offered little protection from the wind. High wind speeds might therefore constitute a limiting factor for bumblebee activity in particularly exposed landscapes. In addition, estimated wind speeds were based on rough observations, and wind gusts were not taken into account. Rough estimations of wind speed, together with a small sample size and masked variations among bumblebee species, might therefore explain the uncertainty of this effect.

#### 4.3 Bumblebee species composition and floral preferences

My results show that floral preferences differed according to bumblebee species. The small-bodied bumblebee species *B. monticola/lapponicus*, *B. jonellus* and *B. pratorum* strongly preferred foraging on *V. myrtillus* in spring. These bumblebee species have previously been reported as abundant visitors to ericaceous species (Yalden 1982, Mayer et al. 2012, Moquet et al. 2015, Moquet et al. 2017b), which could be due to higher foraging efficiency, caused by size matching between short tongues and

shallow nectar tubes (Klumpers et al. 2019). At the same time, small-bodied bumblebees may be constrained by small foraging ranges, leading to stronger dependencies on local resources (Westphal et al. 2006). For example, my constructed network models indicated that *B. monticola/laponicus* preferred to forage for pollen on *Pedicularis* spp. and *A. alpinus*. Flowers of these plant taxa have long corollas (Kwak and Bergman 1996). Nectar resources might therefore be hard to reach for short-tongued bumblebees, leading to decreased foraging efficiency (Klumpers et al. 2019). Thus, small foraging ranges might to some extent explain the diverse composition of plant pollen species in pollen loads from *B. monticola/laponicus*, *B. pratorum* and, *B. jonellus*.

The large-bodied bumblebee taxon *B. sensu stricto* were infrequent visitors to ericaceous species, which is in agreement with foraging behaviour observed in *B. lucorum* and *B. cryptarum* (Scriven et al. 2016). Instead, *B. sensu stricto* strongly preferred pollen of *M. pratense*, which was available in forest habitats at high abundances. Westphal et al. (2006) proposed that bumblebees with large foraging ranges are able to locate rewarding flower patches, allowing large-bodied bumblebees to meet the high energy demands of large colonies. This was corroborated by Mayer et al. (2012) as their findings indicated that large-bodied bumblebees were more sensitive to decreased floral resources and preferred to forage in large plant populations. In my results, *B. wurflenii* additionally showed a strong preference for *M. pratense* pollen, possibly due to adaptations for nectar-robbing on flowers with long corollas (Rasmont et al. 2015).

*B. balteatus* appeared to forage on flowers with deep nectar tubes (i.e., *M. pratense*, *Pedicularis* spp., *A. alpinus*), in the summer, when a higher variety of flowers were available. This is not surprising, given that *B. balteatus* is a long-tongued bumblebee species (Ranta 1982), allowing for a higher foraging efficiency on flowers with deep nectar tubes (Klumpers et al. 2019). Unfortunately, pollen load samples were not collected from *B. balteatus* in summer. Though corbicular pollen loads were not systematically collected, an absence of pollen samples could indicate that *B. balteatus* collected pollen on plant species that were not present in the studied *V. myrtillus* communities. Lastly, *B. polaris* appeared to prefer foraging on *Pedicularis* spp. However, *B. polaris* was rarely observed and pollen preferences were estimated based on one pollen load sample.

#### 4.4 Plant-bumblebee network structure

The degree of network specialization was low in spring, where bumblebee species visited multiple plant species, and plant species were visited by multiple bumblebee species. This is consistent with pollination networks at high altitudes (Ponisio et al. 2016) and in other ecosystems such as urban areas (Baldock et al. 2015), heathlands (Ballantyne et al. 2015), and grasslands (Lucas et al. 2018),

where plant-pollinator interactions formed relatively generalized network structures. The adaptive behaviour of generalist consumers is considered an important function, that buffers and stabilizes population abundances (McMeans et al. 2015), and is arguably fundamental for bumblebees, which remain active during a large part of the year (Waser et al. 1996). Further, network specialization increased with seasonality, indicating that the bumblebee species refined their niche breadth and fulfilled complementary roles in pollen transport. This seasonal increase in network specialization could be caused by an increase in floral richness (Hoiss et al. 2015, Ponisio et al. 2016), suggesting that bumblebees utilize similar resources when floral richness is low, while having the opportunity to forage on more favoured plant species during higher floral richness. Alternatively, competition for floral resources might increase when bumblebee density is high leading to more specialized plant-bumblebee interactions (Brosi and Briggs 2013). On the other hand, Ranta and Vepsäläinen (1981) suggested that spatiotemporal heterogeneity of floral resources constrain bumblebees from utilizing all floral resources within a system, as bumblebee colonies are stationary positioned and spatially distributed within the landscape. Higher floral richness might therefore form heterogenous floral compartments, where plant-bumblebee interactions could be structured by a combination of floral preferences and obligated uses of available resources. Still, the seasonal increase in the degree of network specialization underlines the functional significance of floral resources in spring, which might enhance the survival of spring foraging bumblebees (Carvell et al. 2017) and thus facilitate a knock on effect for entomophilous plant species that bloom later in the year (Blüthgen and Klein 2011).

#### 4.5 Differences in pollen composition of pollen loads

We found no clear difference in corbicular pollen load composition among bumblebee species in spring, which is in agreement with Moquet et al. (2015), and possibly a consequence of the generalized network structure of pollen transport interactions within this period. However, I might not have been able to detect different pollen preferences due to a low sample size of pollen loads in the spring. *B. monticola/lapponicus* collected different plant pollen taxa than *B. sensu stricto* and *B. wurflenii* in summer. These differences were possibly caused by the absence of *M. pratense* pollen in pollen loads of *B. monticola/lapponicus*, while *B. wurflenii* and *B. sensu stricto* collected pollen almost exclusively on *M. pratense*. In addition, no individuals of *B. monticola/lapponicus* were observed visiting *M. pratense*. Interestingly, short-tongued bumblebees can act as nectar robbers before collecting pollen from *M. pratense* flowers (Jennersten and Kwak 1991). It therefore remains unclear why *B. monticola/lapponicus* omitted the use of floral resources offered by *M. pratense*, but can further go to explain the higher degree of network specialization in summer.

Plant pollen compositions clearly differed between pollen loads from spring bumblebees foraging on *V. myrtillus* and *P. lapponica*. Pollen loads from bumblebees collected in the summer showed clear differences between bumblebees foraging on *M. pratense* and *P. lapponica*, and between bumblebees foraging on *M. pratense* and *A. alpinus*. These differences are possibly due to high flower constancy, given these species representation in the majority of monospecific pollen loads.

#### 4.6 Limitations

Sampling in 2018 started approximately on day ten of the *V. myrtillus* flowering season, therefore I cannot be certain that the observed patterns were consistent during this very early period as well. Pan-traps and standardized transects walks are considered more efficient sampling approaches than the observation plot sampling approach we used (Westphal et al. 2008), and more observations could possibly have been recorded using these alternative sampling methods. Consequently, the results of this study are based on a relatively small amount of bumblebee observations. This low number of bumblebee observations necessitated a pooling of all bumblebee species to be able to assess the climatic and floral effects on bumblebee abundance and species richness, masking the potential distinctive behaviour among bumblebee species. However, an observation plot approach was considered appropriate for this study as it enabled the recording of plant-pollinator interactions (Memmott 1999, Reitan and Nielsen 2016) while reducing observer bias (Westphal et al. 2008). Still, some bumblebee individuals may have been repeatedly recorded, but I consider this risk as low since the overall densities enabled us to keep track of the handled individuals within a plot.

Moreover, this study used data from one study area and data sampling was predominantly made within a single season. Therefore, the results may not be generalizable to other areas or time-periods.

## 5 Conclusions

My results demonstrate that occurrences of foraging bumblebees within *V. myrtillus* communities were influenced by both climatic and floral factors. Local temperature had a positive effect on bumblebee abundance and species richness, and the recorded local temperatures, within the range of this study, did not impose an activity barrier for bumblebees. Factors related to preferences for *V. myrtillus* communities in sheltered low alpine environments, which could not be explained by

climatic or floral factors, additionally appeared to influence occurrences of foraging bumblebees. These results highlight the complexities involved in studying bumblebee foraging behaviour. Future research might benefit from incorporating data on nesting sites, in terms of the distribution of colonies, but also with regards to colony success in relation to the surrounding environment. My results further show that the DNA metabarcoding results largely reflected the observed plant-bumblebee interactions, meaning that foraging bumblebees within the forest-tundra ecotone found valuable pollen resources in *V. myrtillus* communities. *V. myrtillus* was particularly important in the spring, where all foraging bumblebees species collected pollen from this species. Under a warmer climate, these potentially mutualistic plant-bumblebee interactions, might accelerate shrub expansions, which could benefit generalist bumblebees at high altitudes. However, upward expansions of tree-lines could decrease the amount of preferred habitats for high-altitude species in the low alpine zone. Long-term research programs or comparative studies will be particularly useful to assess such changes.

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# Appendix

## Appendix 1

Number of observations in plots and number of collected pollen load samples for the identified bumblebee species and OTU's.

Bumblebee species/OTU	Plot observations	Corbicular pollen samples
<i>Bombus monticola/lapponicus</i>	232	34
<i>Bombus wurflenii</i>	30	8
<i>Bombus pratorum</i>	30	7
<i>Bombus balteatus</i>	21	3
<i>Bombus jonellus</i>	17	6
<i>Bombus sensu stricto</i>	8	11
<i>Bombus polaris</i>	10	6
<i>Bombus alpinus</i>	4	-
<i>Bombus pascuorum</i>	2	2
<i>Bombus consobrinus</i>	1	-
<i>Bombus hypnorum</i>	-	1

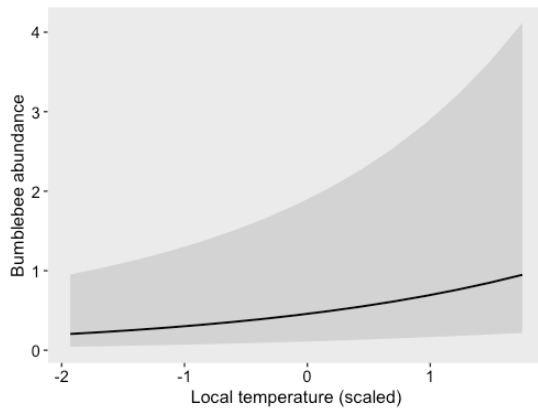
## Appendix 2

Percentages of all recorded flower visits to specific plant species and percentages of the total amount of DNA sequences obtained from DNA metabarcoding of collected pollen load samples. The listed plants are taxa that contributed to more than 1 % of plant pollen taxa in a corbicular pollen load sample.

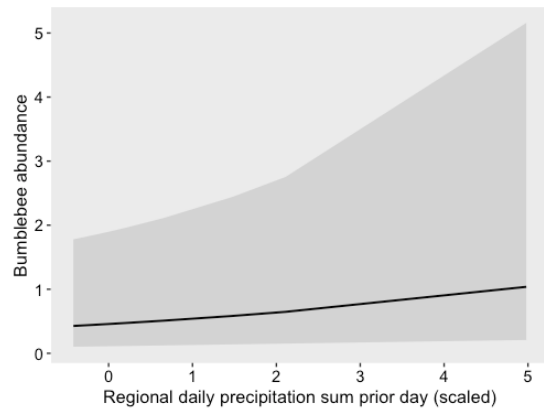
Plant species/taxon	Percentage of observed visits	Percentage of DNA sequences
<i>Vaccinium myrtillus</i>	14.80	36.93
<i>Melampyrum pratense</i>	11.19	22.32
<i>Pedicularis</i> spp.	9.75	23.27
<i>Pedicularis lapponica</i>	-	15.91
<i>Pedicularis oederi</i>	-	7.36
<i>Astragalus alpinus</i>	7.58	6.08
<i>Phyllodoce caerulea</i>	-	5.99
<i>Salix</i> sp.	3.61	0.97
<i>Pyrola</i> spp.	1.44	0.79
<i>Calluna vulgaris</i>	-	0.78
<i>Aconitum lycoctonum</i>	1.08	0.54
<i>Vaccinium uliginosum</i>	14.80	0.47
<i>Scorzoneroides autumnalis</i>	5.05	-
<i>Bistorta vivipara</i>	1.44	-
<i>Saxifraga oppositifolia</i>	0.36	-
<i>Solidago virgaurea</i>	0.36	-
<i>Othilia secunda</i>	-	0.36
<i>Rhinanthus minor</i>	-	0.34
<i>Vaccinium</i> sp.	-	0.19
<i>Trifolium repens</i>	-	0.16
<i>Lotus corniculatus</i>	-	0.13
<i>Saxifraga aizoides</i>	-	0.12
<i>Silene vulgaris</i>	-	0.09
<i>Myosotis</i> sp.	-	0.07
<i>Bartsia alpina</i>	-	0.04
<i>Rubus idaeus</i>	-	0.03
<i>Geranium sylvaticum</i>	18.77	0.02

## Appendix 3

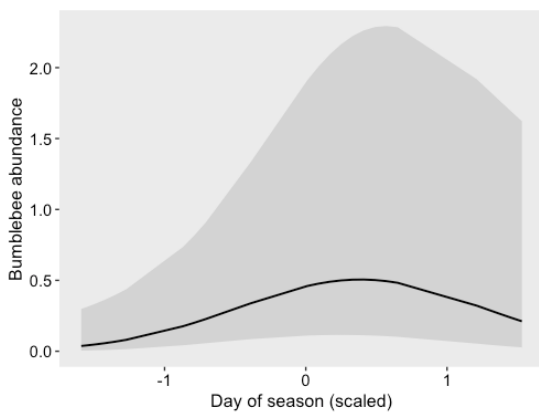
**A**



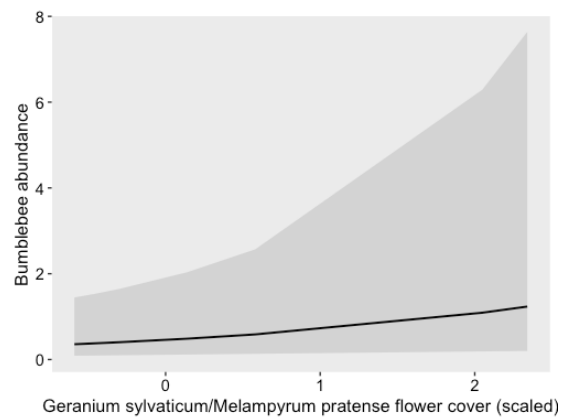
**B**



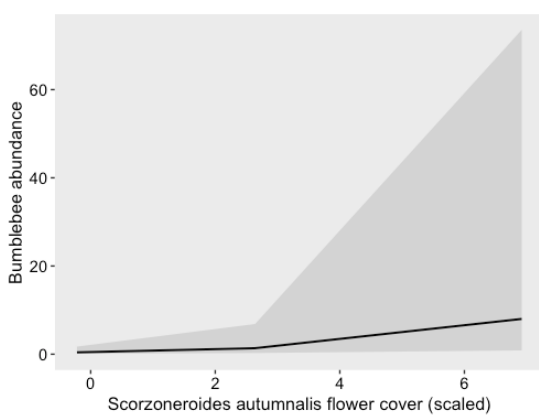
**C**



**D**

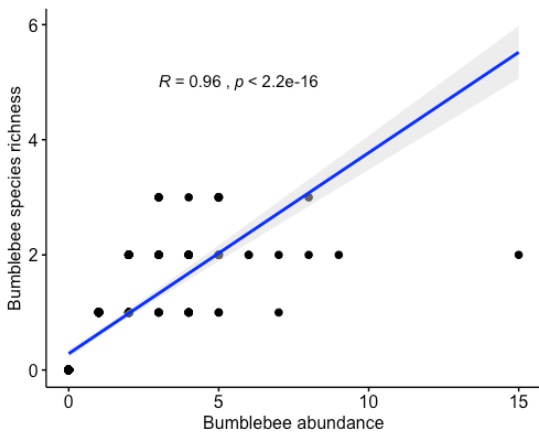


**E**



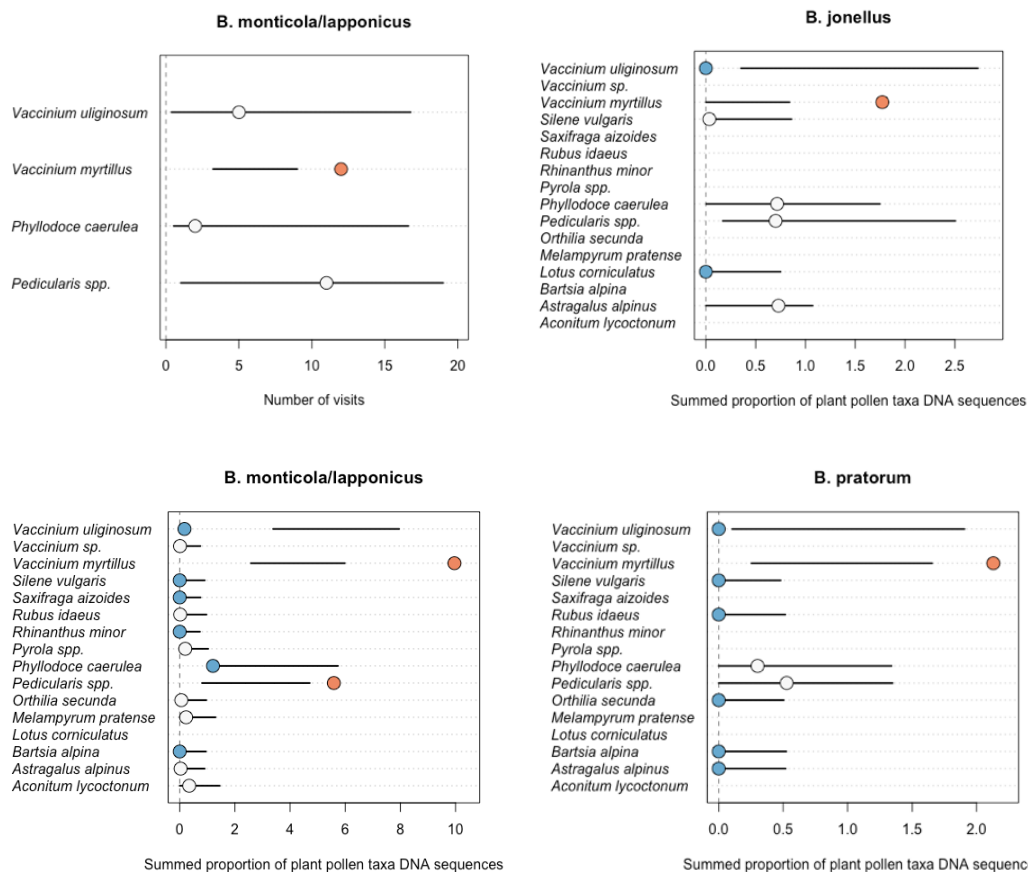
Predicted effects of local temperature (A), regional daily precipitation sum prior day of sampling (B), day of season (C), *G. sylvaticum*/*M. pratense* flower cover (D), *S. autumnalis* flower cover (E) on bumblebee abundance per five minute sampling period at plots.

## Appendix 4



Correlation between bumblebee species richness and bumblebee abundance. The shown correlation coefficient is Spearman's rank correlation coefficient. Values of both variables have been obtained from five minutes sampling at plots.

## Appendix 5

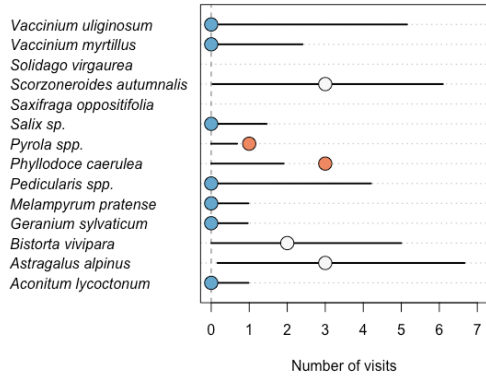


Preference plots for *B. monticola/lapponicus*, *B. jonellus*, and *B. pratorum* in the spring flower visitation network and the spring pollen transport network, comparing the observed number of visits (dots) and the summed proportion of plant pollen DNA sequences (dots) with 95 % confidence intervals from the null models (bars). The orange dot denotes values that were higher than expected under the null model, blue dots lower than expected, and white dots denotes expected values.

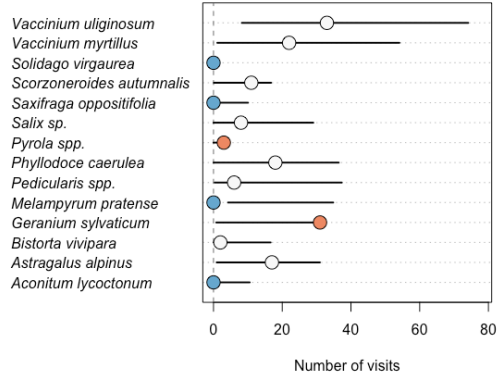


# Appendix 6

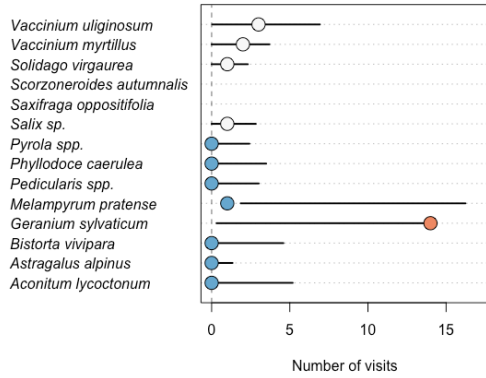
**B. jonellus**



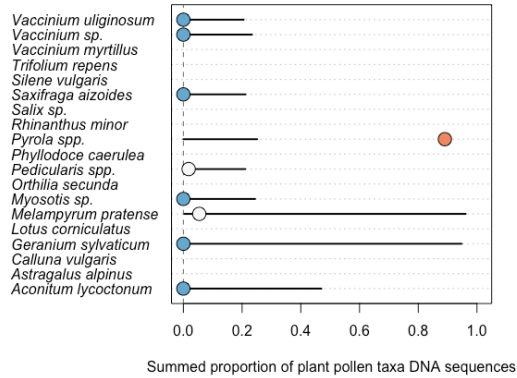
**B. monticola/lapponicus**



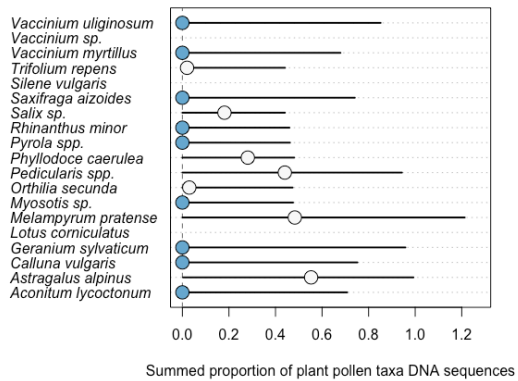
**B. pratorum**



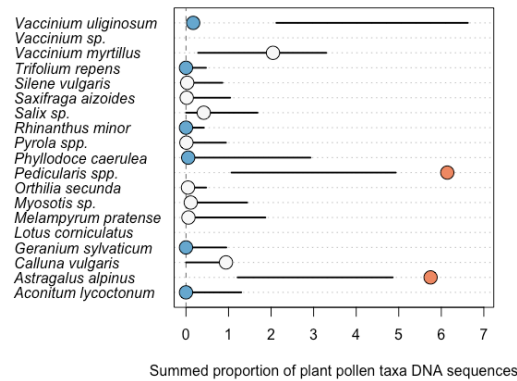
**B. hypnorum**

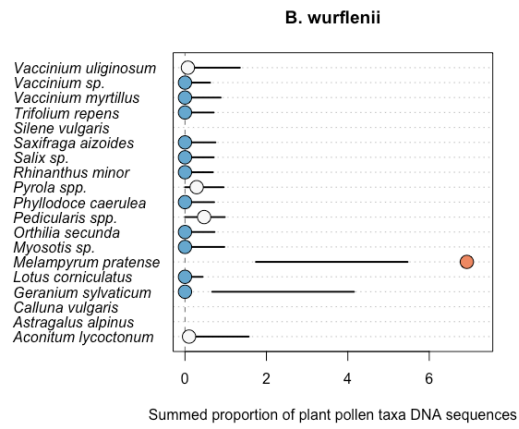
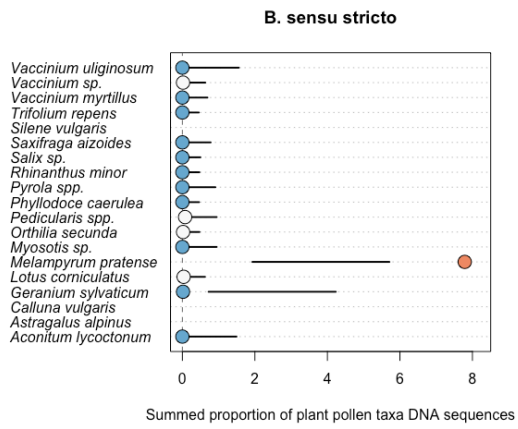
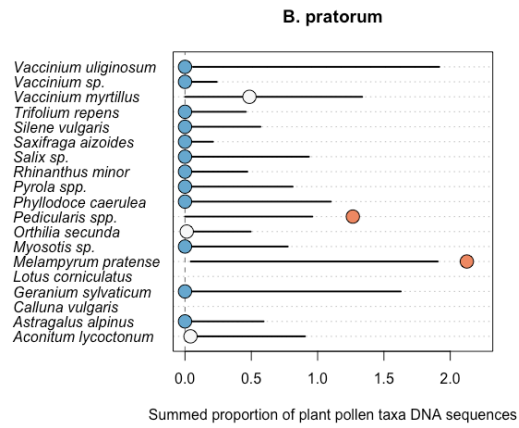
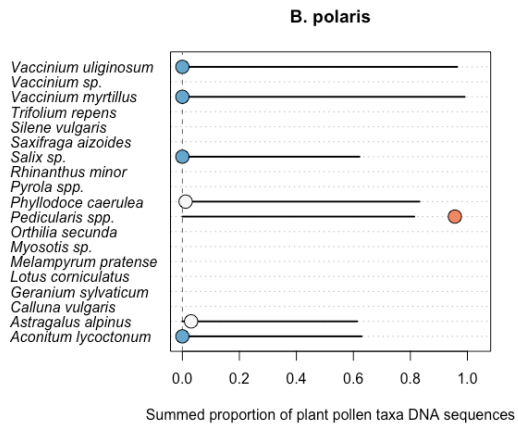


**B. jonellus**



**B. monticola/lapponicus**





Preference plots for *B. monticola/lapponicus*, *B. jonellus*, *B. pratorum*, *B. polaris*, *B. wurflenii* and *B. sensu stricto* in the summer flower visitation network and the summer pollen transport network, comparing the observed number of visits (dots) and the summed proportion of plant pollen DNA sequences (dots) with 95 % confidence intervals from the null models (bars). Orange dots denotes values that were higher than expected under the null model, blue dots denotes values lower than expected, and white dots denotes expected values.

## Appendix 7

Output for perMANOVA carried out to test for differences in corbicular pollen load composition among bumblebee species and among host plant species in spring (df = degrees of freedom, SS = sum of squares, MS = mean square, Pseudo-F = F-statistic,  $R^2$  = coefficient of determination, p-value = significance value). Significant effects are indicated by (\*).

Spring variables	df	SS	MS	Pseudo-F	R2	p-value
a) ~ Bumblebee species + Host plant species						
Bumblebee species	5	1.238	0.248	1.597	0.307	0.114
Host plant species	3	0.627	0.209	1.349	0.155	0.446
Residuals	14	2.171	0.155		0.538	
Total	22					
b) ~ Host plant species + Bumblebee species						
Host plant species	3	1.040	0.347	2.236	0.258	0.040*
Bumblebee species	5	0.825	0.165	1.065	0.204	0.578
Residuals	14	2.171	0.155		0.538	
Total	22					

## Appendix 8

Output for perMANOVA carried out to test for differences in corbicular pollen load composition among bumblebee species and among host plant species in summer (df = degrees of freedom, SS = sum of squares, MS = mean square, Pseudo-F = F-statistic,  $R^2$  = coefficient of determination, p-value = significance value). Significant effects are indicated by (\*).

Summer variables	df	SS	MS	Pseudo-F	R2	p-value
a) ~ Bumblebee species + Host plant species						
Bumblebee species	5	5.546	1.109	9.150	0.563	0.038*
Host plant species	3	1.394	0.465	3.834	0.142	0.122
Residuals	24	2.909	0.121		0.295	
Total	32					
b) ~ Host plant species + Bumblebee species						
Host plant species	4	6.475	1.619	13.354	0.657	0.030*
Bumblebee species	4	0.465	0.116	0.959	0.047	0.385
Residuals	24	2.909	0.121		0.295	
Total	32					