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The effect of *Silene acaulis* on soil bacterial communities across elevational and latitudinal gradients in Scandinavia

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Preface

This thesis is the final part of my master degree in Ecology at the Faculty of Environmental Sciences and Natural Resource Management at the Norwegian University of Life Sciences (NMBU).

I would like to thank my supervisors for their considerable support and guidance throughout this project. To my main supervisor Erik Trond Aschehoug, for his thoughtful advice and for always taking the time talk through any problem. To my co-supervisor Elisa Pierfederici for her help with both fieldwork and bioinformatics, and great conversations.

Further, I want to thank Øystein Bakke for his help and great company throughout the field work at Finse and in Sweden. I would also like to thank Ida Ormaasen for her guidance during the DNA extractions, Aaron DeVries and Vinson Doyle for their help with the library prep, and lab engineer Annie Aasen for helping me with my soil analysis.

Finally, I wish to thank my friends and family for their encouragement and support while working on this project.

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Abstract

Microbes are ubiquitous, extremely diverse, and contribute to important ecosystem functions. Soil bacterial communities, in particular, play an important role in carbon and nutrient cycling, through decomposition and carbon sequestration. In addition, soil bacteria establish mutualistic relationships with plants, animals and other microorganism.

However, soil microbial communities may change in diversity and composition in response to changes in climate, which may disrupt the interactions between soil microbes and other organisms, such as plants. The impact of climate change in arctic and alpine environments is expected to be disproportionately large, thus it is important to understand how soil microbial communities in these environments respond to changes in temperature, precipitation, and plant communities. The alpine cushion plant *Silene acaulis*, is a dominant, foundational species known for its facilitative effects in stressful environments. There have been several studies investigating the facilitative effects of cushion plants on other plants. However, less in known about the relationship between cushion plants and their soil microbiome.

Here, I investigated the effect of *S. acaulis* on its soil bacterial community. I also investigated the effect of latitude and elevation on the soil bacterial communities in rhizosphere of *S. acaulis*, as proxies for climate change. I found that *S. acaulis* had a significant effect on the soil bacterial community composition. Along the elevational gradient, soil microbial communities under *S. acaulis* decreased in richness with increases in elevation. There was a significant effect from latitude on the bacterial composition. However, it appears that site specific factors, such as pH, are more influential along the latitudinal gradient. *S. acaulis* may have a converging effect on the soil bacterial community composition along environmental gradients, and as such, the direct effects of climate change may be weak. Further research is needed to better understand the relative importance of the different factors driving soil bacterial communities in alpine environments.

Contents

Introduction

Soil microorganisms are important drivers of terrestrial ecosystem function (Bardgett & van der Putten, 2014; Fierer, 2017; Singh & Gupta, 2018; van der Heijden et al., 2008). Soil microbes, such as bacteria and fungi, are the main decomposer of organic material, contribute to nutrients cycling, sequester carbon, and establish mutualistic relationships with plants, animals and other microorganisms (Fierer, 2017). Soil bacterial communities make up a large fraction of the below ground biodiversity and have high species richness—one gram of soil may hold several thousand different bacterial species (Torsvik et al., 2002 as cited in Bardgett & van der Putten, 2014). Soil bacteria are also ubiquitous and are found in nearly all terrestrial systems on earth and may even thrive in harsh conditions, such as those found in arctic and alpine environments (Donhauser & Frey, 2018).

Soil microbial communities may also be important determinants of plant community composition and productivity (Bardgett & van der Putten, 2014; van der Heijden et al., 2008). For example, in stressful environments soil microbial communities can help mitigate the environmental stress that plants experience (Lau & Lennon, 2012; Malard et al., 2018). Therefore, the relationship between plants and the soil microbiome may be important for plant growth and survival. Plants are able to influence the microbiome in their rhizosphere by providing a carbon rich habitat and by initiating crosstalk between the plant and the microbes through chemical signaling via root exudates (Bais et al., 2006). This may result in microbes that are beneficial to plant survival and growth to be more abundant. Beneficial microbes may directly contribute to promoting plant growth through the mobilization of soil nutrients, and indirectly as biological control agents protecting the plant against pathogens (Haldar & Sengupta, 2015). Thus, a better understanding of soil microbial community composition may give important insights into overall community function.

Recent developments in molecular methods has made it possible to characterize and map out the functional and taxonomic diversity of entire microbial communities. However, determining the factors that affect the diversity and composition of soil microbial communities still presents a challenge. The main drivers for microbial communities are generally known to be pH and vegetation (Malard et al., 2018). Fierer (2017) found that besides soil pH, more specific variables driving the microbial community structure were: organic carbon quality and quantity, nitrogen availability, temperature, and redox status. Carbon availability, in particular, may

provide a predictive framework for which bacterial taxa may be found in different habitats (Adamczyk et al., 2020; Chen et al., 2021; Fierer et al., 2007; Yao et al., 2017). Microbial taxa considered to be copiotrophs – *Firmicutes, Alpha-* and *Beta-proteobacteria, Bacteroidetes* and to some extent *Actinobacteria* – (Adamczyk et al., 2020; Fierer et al., 2007; Francioli et al., 2016) will be found in higher abundance in soil with higher carbon availability (copiotrophic habitats) and in lower abundance in soil with low carbon availability (oligotrophic habitats). In contrast, microbes that are considered to be oligotrophs – *Acidobacteria, Verrocomocrobia, Planctomycetes* and *Chloroflexi* (Bergmann et al., 2011; Davis et al., 2011; Kaboré et al., 2020) will be found in high abundance in low carbon availability soils, and in low abundance in high carbon availability soils. In alpine soils for instance, the access to organic matter is often restricted due to low plant productivity. Therefore bacterial communities in alpine zones needs to adapt to oligotrophic, nutrient limited conditions (Donhauser & Frey, 2018). The relationship between microbes and plants in environments such as arctic and alpine tundra may therefore be essential for the microbial community, as plants may for example provide the microbes with carbon, water, and protection against UV.

Due to the importance of microbial communities in ecosystem functions, changes in diversity, community composition, or decreases in abundance may have severe consequences (Hutchins et al., 2019). Microbial diversity is strongly affected by changes in temperature and precipitation, as well as indirect effects due to changes in plant growth and community structure. Therefore, the effects of climate change on microbial ecology is likely to be highly significant (Classen et al., 2015). Both arctic and alpine areas are disproportionally affected by climate change, as the temperature in these areas are increasing more than the global average (P.R. Shukla, 2022). Obvious changes have already started to be visible, with species shifting towards higher altitudes and latitudes and increased greening in both alpine and arctic environments (Wookey et al., 2009). However, due to the difficulty of accessing and working in alpine and arctic regions, the dynamics between plants and their soil microbiome in these areas is not well understood (Donhauser & Frey, 2018).

Cushion plants are a highly adapted, dominant plant form in alpine and arctic environments. They form a dense dome of leaves over their roots which creates a small microhabitat that traps litter and soil into a closed nutrient cycle. These “nutrient-traps” (Korner, 2021), also create favorable habitats for decomposers. Cushion plants also capture a significant amount of heat inside the dome of leaves, making them efficient heat-traps as well (Korner, 2021). The ability of cushion plants to create microhabitats that are relatively less stressful also promotes

facilitation of other plant species (Antonsson et al., 2009; Badano & Cavieres, 2006; Callaway et al., 2002). Thus, cushion plants have important impacts on both above and below ground community structure and are considered foundational species within arctic alpine zones (Badano & Cavieres, 2006).

Silene acaulis is an alpine cushion plant that has a widespread, circumboreal distribution in the northern hemisphere (Mossberg et al., 2018). Several studies have investigated the relationships between cushion plants and their soil microbiome (Rodríguez-Echeverría et al., 2021; Wang et al., 2020), including *S. acaulis* (Ciccazzo et al., 2014; Massaccesi et al., 2015; Roy et al., 2013; Roy et al., 2018). Cushion plants have, in general, been found to have a significant effect on the structure of the soil microbial community. To understand how the relationship between cushion plants and their soil microbial communities may change due to climate change, elevational and latitudinal gradients have been used as a proxy for changes in temperature. Roy et al. (2013) found that *S. acaulis* had a converging effect on its soil bacterial communities along elevational gradients in certain habitats (Roy et al., 2013). Rodriguez et al. (2021) found that one of the main influences on the soil microbiome along a latitudinal gradient was the presence of the cushion plant genus *Azorella* but the effect of *Azorella* was smaller in sites experiencing higher environmental stress.

Here, I explored the effects of *Silene acaulis* on soil microbial communities along both an elevational and latitudinal gradient. I used molecular methods to extract environmental microbial DNA from soil collected either from under *S. acaulis* or from nearby unvegetated, bare soil. Further, I sought to understand the relationship between *S. acaulis* and the bacterial communities in its rhizosphere. Including which factors drive the diversity and composition of the soil microbial communities over environmental gradients.

I hypothesize that (1) the soil bacterial community under *Silene acaulis* is significantly different in composition compared to the soil bacterial communities in bare soil (Roy et al., 2013; Roy et al., 2018; Wang et al., 2020); and (2) changes in both elevation and latitude significantly impacts soil bacterial community composition (Ren et al., 2018; Roy et al., 2013; Yao et al., 2017).

Methods

Study species

Silene acaulis is a common, perennial alpine cushion plant in the family Caryophyllaceae. With a circumpolar distribution and can be found in both xeric and mesic areas, often in calcareous soil (Mossberg, 2018). *S. acaulis* is considered a foundational species due to its ability to facilitate other species and increase diversity in alpine zones. (Badano & Cavieres, 2006). *S. acaulis* is slow growing and long-lived; the oldest individual has been recorded to be over 300 years old (Korner, 2021).

'Figure 1. Photo of *Silene acaulis.* Finse, Norway, July. 2021

Study area

I sampled during August 2021 from seven different populations of *Silene acaulis* across seven sites at three different locations along a latitudinal gradient from Finse, Norway (60°N) to Åre, Sweden (Jämtland; 63°N) and Abisko, Sweden (68°N). The sites in Sweden were previously established (ca 2011) as part of a long-term demography study on *S. acaulis*. In addition, at Finse I established an elevational gradient, with three populations along a transect of 193 m

change in altitude, from 1357 to 1550 masl. The habitats in all locations are characterized as alpine tundra.

Figure 2. Map of sampling locations along a latitudinal gradient in Norway (Finse) and Sweden (Jämtland and Abisko). From geonorge.no (2022).

Abisko

I utilized two previously established study sites at 68°N near Abisko, Sweden. The average temperature for the growing season in Abisko is 7.2℃, and the average precipitation for August is 0.86 mm (1990-2021; SMHI.se). Both sites have exposed phyllite bedrock (sgu.se, 2022) and vegetation cover is scarce. Site one (SA1) consists of two transects and is located just below the top of Loktacohkka on the north facing side at 1122 masl. (68°24’18.4”N 18°21’25.2”E). Site two (SA2) has one transect and is located below the tops of Njulla and

Slottatjokka at 1141 masl. (68°21’56.1”N 18°40’42.7”E) and lies on a slight slope facing northwest. This site is slightly more wind exposed than site SA1. The soil samples from under *Silene acaulis* at SA1 were characterized as loam, whereas at SA2 they were characterized as clay loam. The samples of bare soil from SA1 were sandy clay loam and sandy clay at SA2.

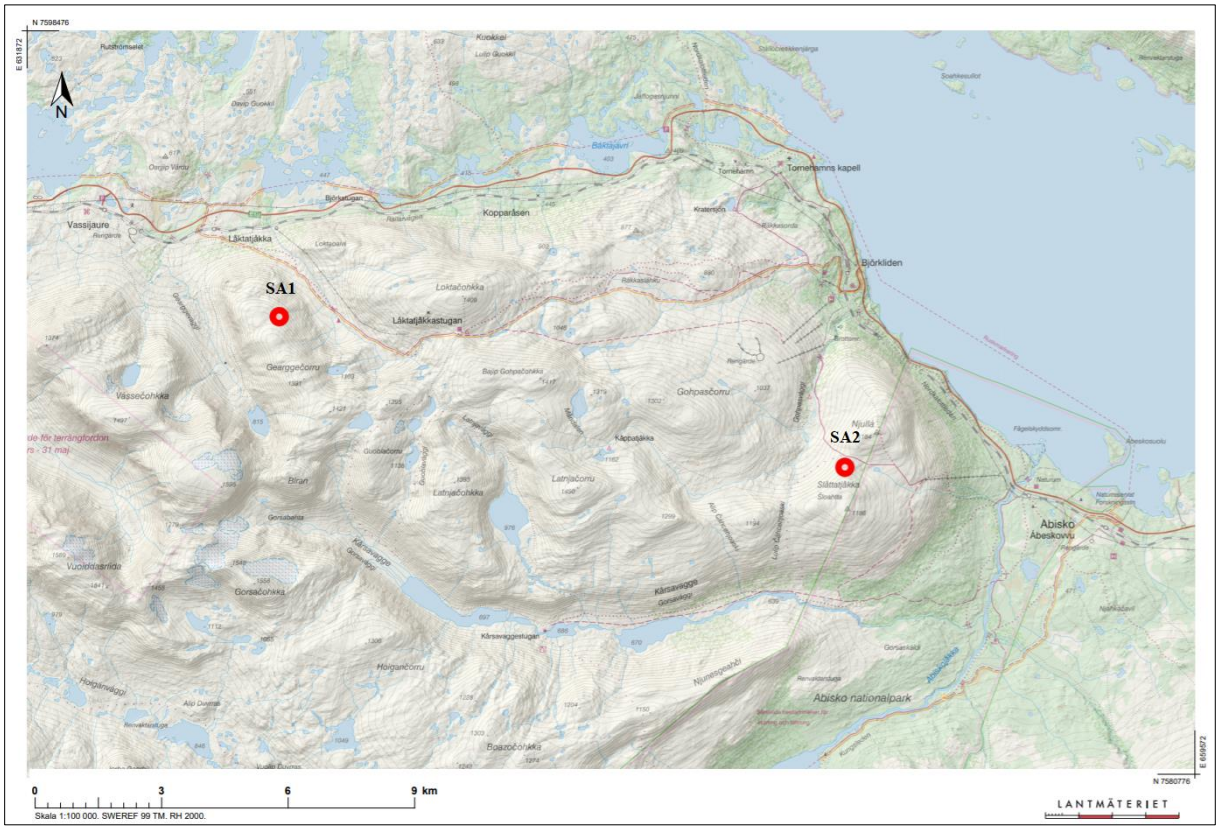

Figure 3. Map over the sampling locations SA1 and SA2 in Abisko. From lantmateriet.se (2021).

Jämtland

The sites in Jämtland are situated at a latitude of 63 N between the tops of Tväråklumparna and were established in 2011. The two sites SJ1 and SJ2 are separated by about 300m. The average temperature for the growing season is 6.62℃ measured between 2010-2021 from Blåhammaren-A weather station (SMHI, 2022), and the average precipitation is 2.26mm measured between 1990-2019from Storlien weather station. Site SJ1 consists of two transects at 1162 masl. (63°12’19.9”N 12°20’18.3”E). Site SJ2 has two transects and lies at 1189 masl. (63°12’15.5”N 12°19’47.9”E). The bedrock here consists mainly of gneiss (SGU, 2022), and as in Abisko, the topsoil layer is very shallow with exposed rocks and bedrock. The soil under *S. acaulis* at SJ1 was characterized as loam, and at SJ2 as silty loam. The bare soil at both SJ1 and SJ2 were characterized as clay loam.

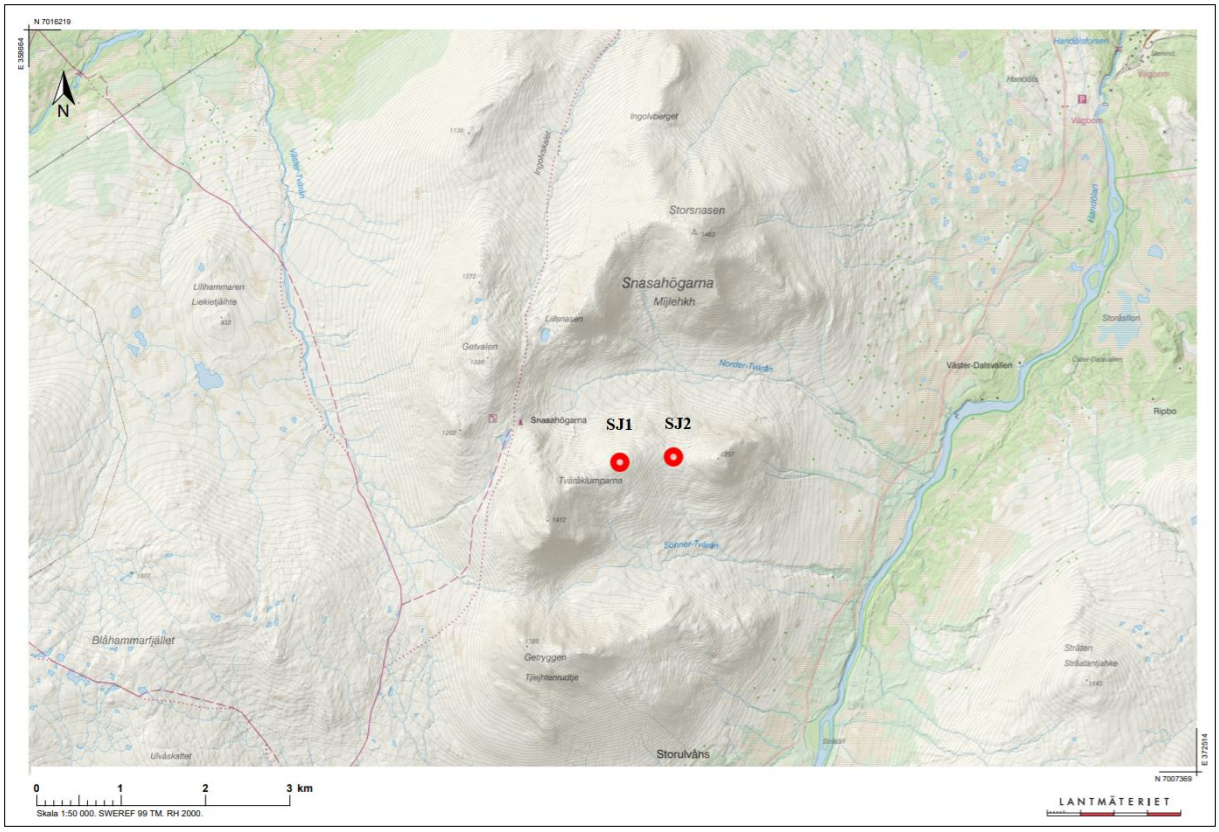

Figure 4. Map over the sampling locations SJ1 and SJ2 in Jämtland. From lantmateriet.se (2021).

Finse

At Finse, I established sampling locations within three populations across an elevational gradient. The average temperature for the growing season at Finse is 5.7℃, and the average precipitation is 2.7mm, measured between 1993-2021 from Finsevatn weather station (MET, 2022). The populations lie at a latitude of 60°N, and the transect starts at an altitude of 1357 masl. (60°36’44.9”N 7°30’27.0”E), the mid population at 1455 masl. (60°36’48.6”N 7°31’08.1”E) and the highest population is situated at 1550 masl. (60°36’57.6”N 7°31’21.1”E; Figure 5). The bedrock across this transect is mainly phyllite, schist and in places calcareous or with layers of limestone (NGU, 2022).

The lowest site lies close to a trail and may therefore be more exposed to human disturbance and grazing sheep than the other sites. The soil sampled from under *Silene acaulis* was characterized as silty loam, and the bare soil at this site was characterized as clay loam. The mid site lies on a ridge on the southwest facing slope of Sanddalsnuten, this site’s vegetation is similar to the lower but here the topsoil layer here is shallower. The soil under *S. acaulis* was characterized as sandy loam and the bare soil was clay. The highest site is found at the top of

Sanddalsnuten, which is highly wind exposed. The soil under *Silene acaulis* is characterized as silty loam, and the bare soil as loam.

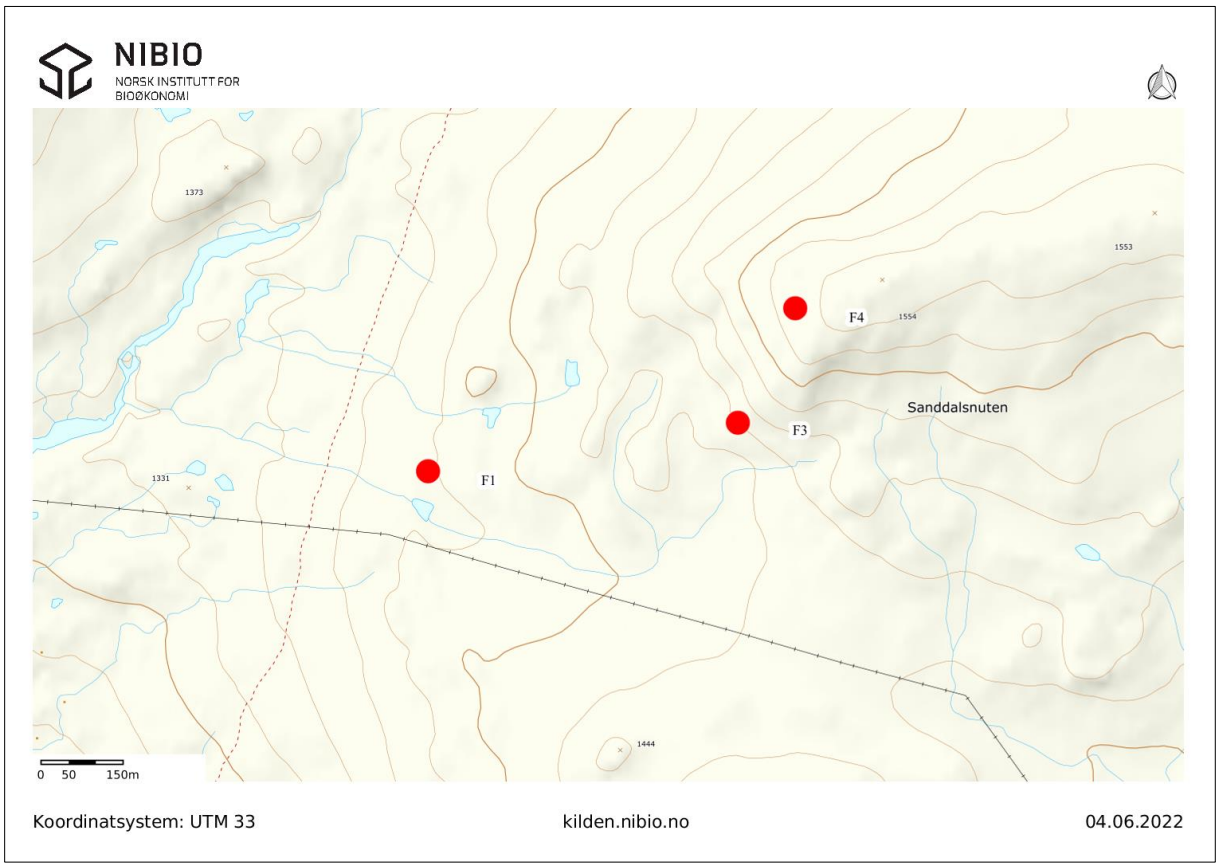

Figure 5. Map over the elevational transect in Finse. Where F1 is the population at the lowest altitude and F4, located on the top of Sanddalsnuten, is the highest. From Kilden.nibio.no (2022).

Data collection

Temperature data

TOMS dataloggers were used to collect data on sub-surface soil, soil surface, and air temperature, as well as soil moisture from July 2020 to July 2021 at all sites except F1 and F3. Readings were taken every 15 minutes. At site F4, four TOMS dataloggers were deployed around the site, while at sites A1, A2 and SJ1 and SJ2, two TOMS dataloggers were deployed at each site. Additional climatic data was gathered from the closest weather station to each site.

Characterizing Silene acaulis populations

I measured the cushion area and reproduction of a subset of individuals for each of the seven populations of *Silene acaulis.* Area was calculated by measuring the maximum size ellipse of each cushion and estimating the percent area missing to account for the irregular shape as described in (Doak & Morris, 2010). Reproduction was measured by counting the number of mature seed capsules on each individual.

At Finse, I measured plants from three populations along the elevational transect. All populations were chosen by creating plots of 50m by 50m with a minimum of 50 individuals of *Silene acaulis* within these plots. Within each population, individuals were chosen by establishing a 25m transects and measuring all *S. acaulis* within an approximately 1m band on either side of the transect. I measured 43 individuals at the lowest elevation site (F1), 28 individuals were measured at the mid-elevation site (F3), and 28 individuals were measured at the high elevation site (F4). In all four locations in Sweden the transects varied between 30-50m and were established in 2011-2012 as part of a long term demography study. At site SJ1 and SJ2, 222 and 166 individuals were measured respectively. I measured 175 plants at site SA1 and 172 at site SA2 in Abisko.

Soil sampling

At each site I collected soil samples from the rhizosphere of five *Silene acaulis* individuals and one from bare soil as a control. To insure that individual *S. acaulis* were well established, I only sampled from individuals that were larger than 10 cm in diameter. Otherwise, individuals were randomly selected at the Finse sites. In Sweden I preferentially sampled soil from the rhizosphere of individual plants that are included in a multi-year seed endophyte study to allow for future comparisons between soil microbial communities and individual seed endophyte communities.

For all samples, I collected 2-3 replicates with a sterilized metal corer 1.5 cm in diameter. Replicates were combined and homogenized in a 50 mL sterile conical tube (hereafter "tubes"). The number of replicates depended on the abundance of soil underneath the plant. After each set of replicates, the corer was washed with water and sterilized with 70% ethanol and a small handheld burner. When sampling I used gloves to avoid contamination from handling and possible cross-contamination between the samples.

The tubes were placed in a cooler with icepacks on site and continuously stored in 5 ℃ after sampling and during transport from the sites to the lab. The samples were stored at 5 ℃ for no more than 4 days in total. Once at the lab, tubes were placed in a –80 ℃ freezer prior to processing

Soil Analysis

Samples were thawed site by site to insure the handling time was as low as possible during processing. Each individual soil sample was homogenized using a beadbeater, then sieved using a 2mm sieve in preparation for DNA extraction. I subsampled 3-4 mL of the processed soil and stored it in a 5mL eppendorf tube in a –20 freezer while waiting to be freeze-dried. This was done on a sterile bench, and all tools where sterilized with ethanol and a Bunsen burner.

The freeze drying was done at -52℃ and 0.129 mBar. I made a small hole in the top of the tube and the procedure took 24 hours until it was completely dry, the samples were then stored in a –20 ℃ freezer before DNA extraction. The rest of the sampled soil I used to measure pH and water content. I measured pH with a pH-meter in a dH2O and soil suspension, of a 1:2.5 ratio. Since the soil was wet, I measured the pH after just 10 min to make sure that the pH did not change due to microbial activity.

The soil texture were estimated by using a field protocol for soil characterization (*Soil Characterization Protocol*, 2014). The water content was measured gravimetrically by weighing wet unsieved soil, drying it in a drying oven at 105 ℃ for at least 24 hours (until stable) and weighing the dry soil again. To calculate the water content I used this equation:

$$\frac{mass\ of\ moist\ soil\ (g) - mass\ of\ ovendried\ soil\ (g)}{mass\ of\ ovendried\ soil\ (g)} \times 10$$

DNA-extraction and sequencing

I used a standard metabarcoding method to get bacterial taxonomic information about the soil microbial community. Metabarcoding uses marker gene amplification to identify the organisms found in the sampled environment. It targets specific regions on specific genes that vary between taxa (Taberlet et al., 2012). These genes consist of variable regions that makes it possible to infer phylogenetical relationships between taxa. Between these variable regions lies

segments that are highly conserved, making these regions perfect as binding sites for PCR primers (Knight, 2018). The gene that codes for the 16s ribosomal unit is the most commonly used for metabarcoding bacteria. This method gives a high-level overview of the bacterial community in the sampled soils. Making it possible to characterize bacterial communities, including the diversity and composition of the community, but at low resolution (Knight, 2018).

DNA-extraction

The DNA extraction was performed using DNeasy Powersoil pro kits from Qiagen. All extractions followed the protocols provided by the kit manufacturer. The bead-beating was done with the Fastprep 24, 4.5 m/s for 45s. I quantified the extracted DNA with a qubit fluorometer. The quality was assessed with gel electrophoresis on a subset of the samples, two per site using 1% agarose gel and 1g agarose in a 200 mL TAE-buffer. I also introduced negative and positive controls during this step. The extracted DNA was then dried down with a speedvac for transport. I sealed each microtube with parafilm and the samples were shipped to Louisiana State University, USA for PCR library prep.

PCR procedure and sequencing

A nested PCR approach was used to amplify 16S regions of the extracted DNA, three technical replicates of the first PCR reaction was done, each performed on different days and machines using amplicon-specific primers and high DNA concentrations (10ng).
The products were then used for PCR2 which attached indexed Illumina adaptors. Per library all reactions were then pooled into a 1.5ml tube to create a single indexed library. The products were checked on a gel so that the relevant band could be cut out and extracted to eliminate fragments. The resulting DNA where checked for quality and quantity with nanodrop and HS Qubit assays. The libraries where then pooled and a volume at a concentration of 10nM/µl was prepared and shipped for sequencing.

The primers used were the 515F-Y (GTGYCAGCMGCCGCGGTAA) and 805R (GACTACHVGGGTATCTAATCC), which encompasses the V4 region. The sequencing was done on an Illumina MiSeq v2 platform by Admera Health, with 2x250bp paired end reads and a sequencing depth at 24-30M reads. Both libraries were done at the same time in one run with

20-35% PhiX. Due to an issue at the cleaning step during library prep, the sequencing had to be done with a 10 fold higher concentration than normal.

Bioinformatics

I received paired end reads in a Fastq format; each amplicon came in two files one for forward reads (R1) and one for reverse reads (R2). I did the bioinformatical analysis mainly using R Statistical Software (v4.1.2; R Core Team 2021) and one step using cutadapt version 2.8 (Martin, 2011).

I started to investigate the reads and trim the primers with cutadapt, here I found that there were many reads which had nonmatching primers to tags. Because the samples were prepped for both 16s and ITS sequencing, when trimming the reads I discarded the untrimmed to make sure I did not have any reads from the ITS library, which is not the focus of this thesis. The trimmed dataset was loaded into R for further processing.

For denoising and clustering I used the DADA2 pipeline, specially developed for microbial communities (Callahan et al., 2016). Error models can be found in the appendix (Appendix figure 1 and 2). When assigning taxonomy I used the RDP trainset 18/release 11.5 and bootstrap level 80, as recommended by Callahan et al. (2016) for genus level. I did a cross reference with BLAST using the NCBI database on the most abundant ASVs, to make sure that the taxonomic assignment gave a similar output at genus level.

The finished ASV table and taxanomic table was esported together with metadata from the sample sites into a phyloseq object with the R package phyloseq for further analysis.

I used decontam to test for and remove possible contamination (Davis et al., 2018), for this the negative controls from the extraction were used in the frequency method of the decontam package. Here 75 out of 9655 ASVs were removed from the data.

Statistical analysis

Preprocessing in R

The dataset was further filtered by keeping reads with a prevalence > 5%, and total count per ASV >= 50, which retained 1356 ASVs out of 9582. This threshold was decided when

investigating the data by prevalence and abundance per phylum. The reads were normalized by transforming them into relative abundance, based on downstream analysis methods. A rarefaction curve (Appendix figure 3) identified some samples with very low samples sizes and few species, this was especially notable for sample 35. I plotted the whole dataset into an NMDS ordination to see how the data clustered and found that sample 35 (s81 in rarefaction curve plot) did not cluster with the other samples well. I found that this sample had a high amount (~20%) of tag jumps, it was therefore removed from the dataset.

Analyzing diversity

I calculated alpha diversity for all samples which were divided into three groups: Control sample vs plant soil sample, elevation vs elevation at Finse, and latitude vs latitude (consisting of the sites F4, SJ1, SJ2, SA1, SA2). I used the vegan package to calculate the richness and diversity using the Chao1, ACE index (calculated with the untransformed dataset), and Shannon diversity index (calculated with the normalized dataset) for all groups. The diversity index was tested with Analysis of variance (ANOVA) for significance within the groups and the richness was tested with a Kruskal-Wallis test due to its non-normality. For the groups with three levels I did a post hoc test using a pairwise Wilcoxon test (Joos et al., 2020).

A Non-metric multidimensional scaling (NMDS) ordination of Bray Curtis distances was calculated to analyze the beta-diversity between the samples within the groups. The stress was tested for each of the three ordinations and all stress levels were below 0.15 for all groups at D=2 (Paliy & Shankar, 2016). I tested the difference between the groups of interest with a Permutational multivariate analysis of variance (PERMANOVA) using the adonis2 function from the vegan package. The assumption of homogeneity within the tested variables was tested with the function betadisper, also from the vegan package, to make sure the results from the PERMANOVA could be trusted. The betadisper results were not significant. A pairwise comparison between the different levels of latitude and elevation was done with a post hoc test. I tested variables for collinearity against each other, excluding variables which would give false significance. To find the factors best explaining the variance in the data I tested candidate models with PERMANOVA, using adonis2 and a step-wise procedure to find the best fitting model. I only tested variables which had a significant difference between the groups of interest.

Relative and differential abundance analysis

The average relative abundance was calculated as the proportion of a taxon within each group: plant soil samples, control samples, low elevation, mid elevation, high elevation/low latitude, mid latitude, high latitude. The relative abundance was displayed using the microeco package.

I tested the differential abundance between the groups using the DESeq function in the DESeq2 package. The pairwise comparison was conducted by using Wald test and a significance threshold of alpha = 0.05. I adjusted the P-values with the Benjamini and Hochberg correction method and selected a false discovery rate at 10% (Love et al., 2014).

Analysis of biotic and abiotic data from study site

All metadata from the sites and soil analysis were added into a single data frame with R. I tested the effects of sample type, elevation and latitude on all of the factors sampled. Before I tested the significance with an ANOVA, all factors were tested for the assumption of homogeneity of variance using a Levene test from the package car, and I checked the assumption of normality using a histogram of the distribution.

Factors used in the analysis: mean proportion of seed capsules per cushion area, mean cushion area, mean pH, mean gravimetrical water content, mean air temperature, mean soil temperature, mean precipitation, mean soil moisture.

Cushion area expressed in cm^2 violated the assumption of normality, therefore I used a logistic transformation on this variable.

I tested the relationship between the cushion area and the number of seed capsules, to test if there is a correlation between size and number of capsules. I analyzed the number of seed capsules as a response variable with cushion area, latitude, and for the Finse samples, elevation, as predictor variables. The number of seed capsules per cushion area was calculated with a general linear model. Here I used a quasi-poisson distribution as the count data of the seed capsules were heavily over dispersed, the overdispersion was tested by with the function dispersion test from the AER package in R.

All plots were made using the ggplot package.

Results

Characterization of the study-area and soil properties

Plant soil vs control soil samples

I found no significant difference in pH between soils collected from under *S. acaulis* compared to bare, unvegetated soils (Figure 6). However, there was a significant difference in the gravimetrical water content measured in the lab where the soil sampled from the plants had a water content of 107.8 g g^{-}, and the control samples had an average water content of 36.1 g g^{-} ($p = 0.00023$).

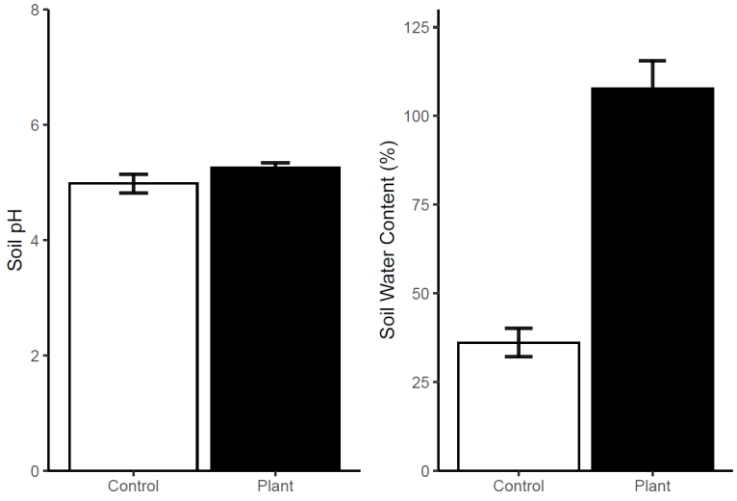

Figure 6. Mean pH and water content, compared between control samples and plant soil samples. The error bars represent +/- SE.

Elevational gradient at Finse

Between the three populations in Finse I found no significant difference in average pH or the mean proportion of seed capsules per cushion area. I also found that the population at the highest elevation had, on average, significantly higher water content in the samples than samples from the lower elevations (Figure 7).

Mean cushion area in the lowest elevation population was significantly smaller than that of mid and high elevation populations (Figure 7). The relationship between the cushion area and number of seed capsules was positively correlated (Figure 8).

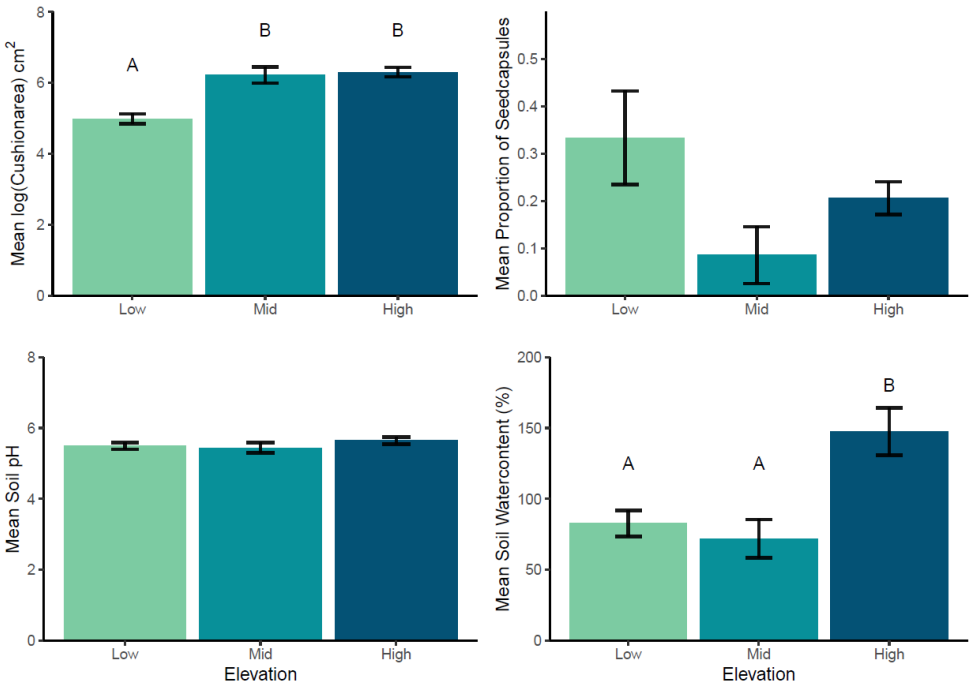

Figure 7. Factors measured per population on the three different elevations in Finse. Significant level < 0.05. Season in this plot refers to this year's growing season. The error bars represent +/- SE.

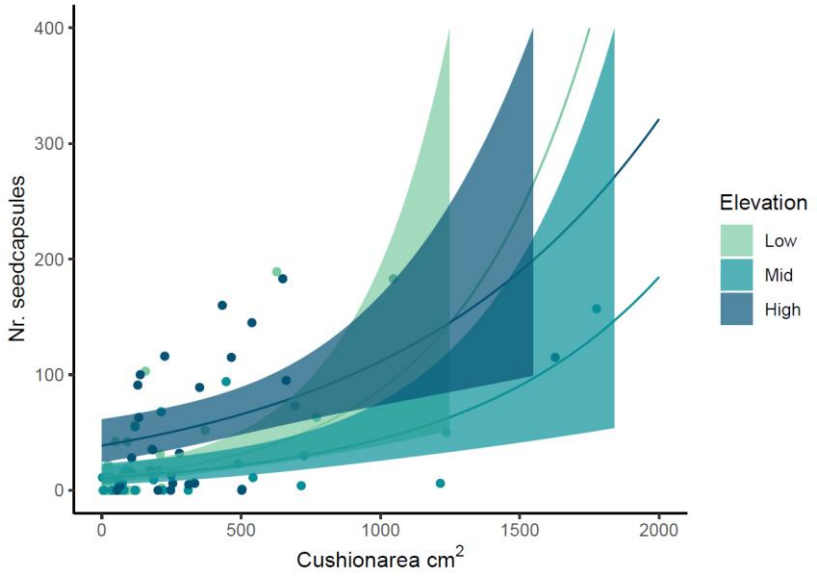

Figure 8. GLM of the relationship between the cushion area of *Silene acaulis* and the nr of seed capsules per site in Finse.

Latitudinal gradient

I found significant differences across the latitudes for all factors except average water content of soils (Figure 9). I found here that average cushion area is biggest at the lowest latitude then the two higher latitudes (Low = 5.6 cm^2(log), Mid = 3.5 cm^2(log), High = 3.3 cm^2(log)). I calculated the proportion of the number of seed capsules per cushion area and found that this was significantly lower at the mid latitude compared to both the low and the high latitudes (Low = 0.196, Mid = 0.03, High = 0.11). Overall, the cushion area and number of seed capsules show a significant positive relationship, suggesting that these two factors are positively correlated (Figure 10).

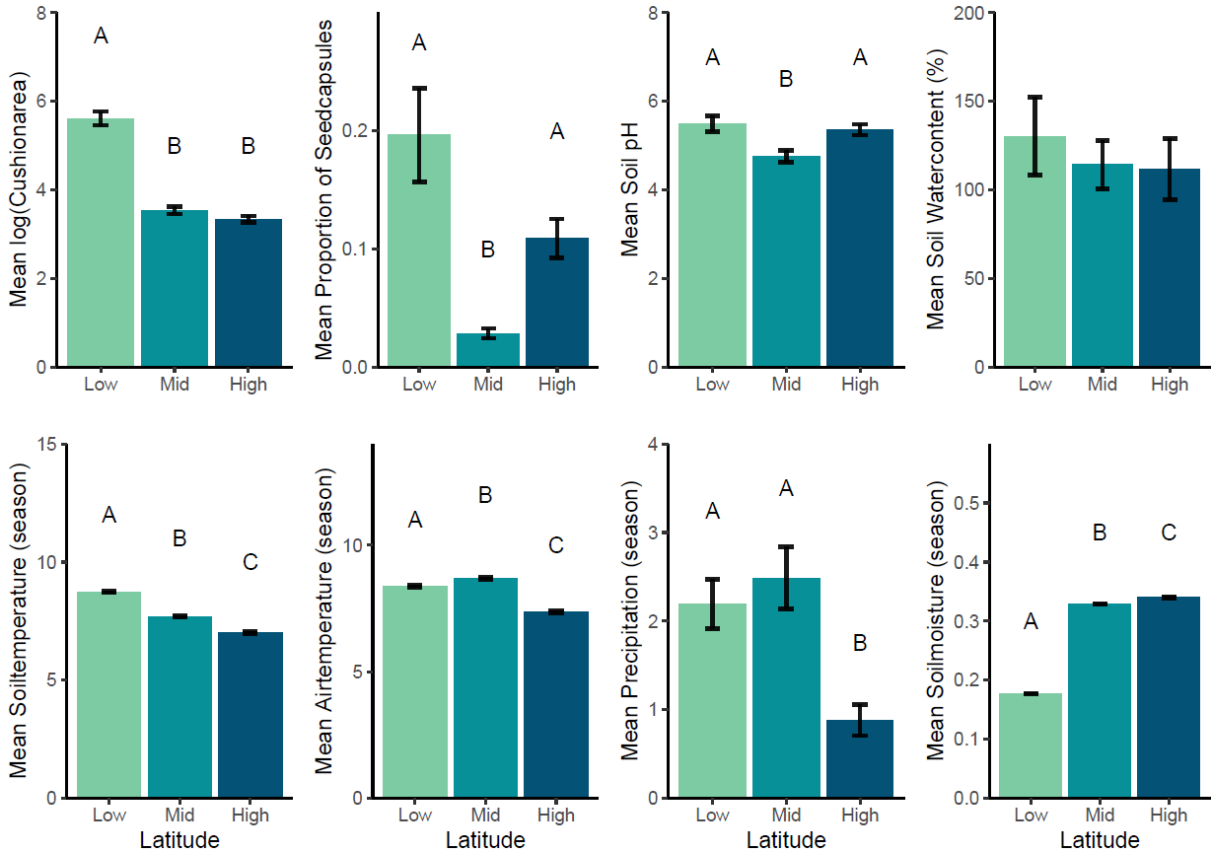

Figure 9. Factors measured on each site over the latitudinal gradient, here representing the top population in Finse and all populations in Jämtland and Abisko. Significant level < 0.05. Season in this plot refers to this year's growing season. The error bars represent +/- SE.

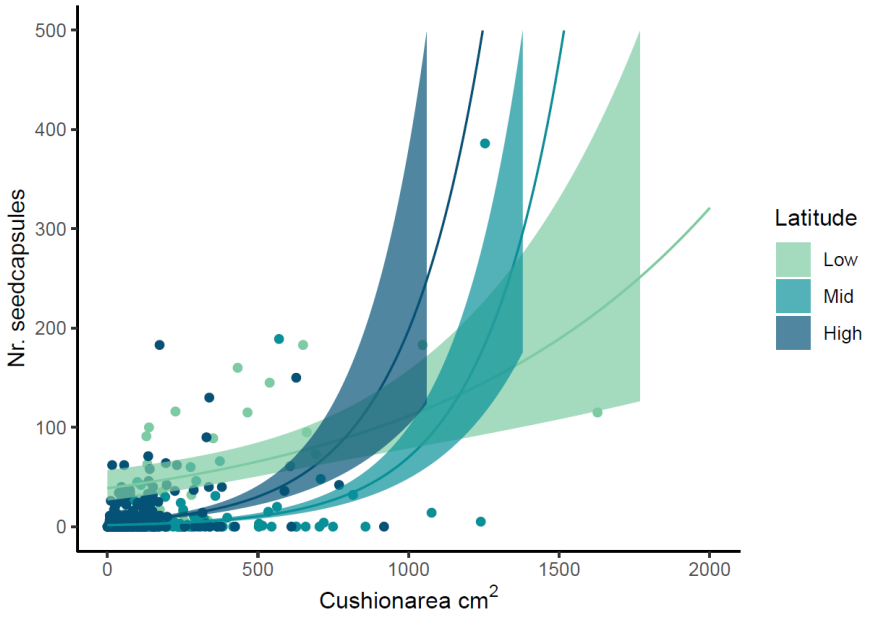

Figure 10. GLM of the relationship between the cushion area of *Silene acaulis* and the nr of seed capsules per latitude. Also here representing the top population in Finse and all populations in Jämtland and Abisko.

The sites at the highest latitude were found to be the coldest sites between the three latitudes during my sampling season (Soil temperature: Low = 8.7°C, Mid = 7.7°C, High = 7°C, Air temperature: Low = 8.4 °C, Mid = 8.7°C, High = 7.4°C), as well as having the lowest average precipitation (Low = 2.2 mm, Mid = 2.5 mm, High = 0.9 mm). However, the driest site was found at the lowest latitude when the average soil moisture was measured (Low = 0.17, Mid = 0.33, High = 0.34). The average pH measured at all sites showed a significantly lower pH level at the mid latitude compared to the high and low latitudes (Low = pH 5.5, Mid = pH 4.8, High = pH 5.4).

Effects of *Silene acaulis* on its soil bacterial communities

Diversity of the bacterial communities in plant soil- and control samples

After filtering out taxa with total abundance lower than 50 and prevalence lower than 5% the resulting dataset used for comparing plant soil- and control samples contain 1356 unique reads from the processed data. The full dataset consists of all in all 74 bacterial families.

ANOVA comparisons of the Shannon index per sample and richness indicated no significant differences between plant soil- and control samples.

However, the analysis of the beta-diversity revealed a significant difference between the bacterial communities from the plant soil samples and the control samples (Figure 11a). An NMDS ordination of the data shows the centroids of control and plant soil samples on different sides of the zero point along both axes in the plot, which suggests the two communities differ in beta-diversity. However, the two groups have a strong overlap within in the NMDS ordination (Figure 11b).

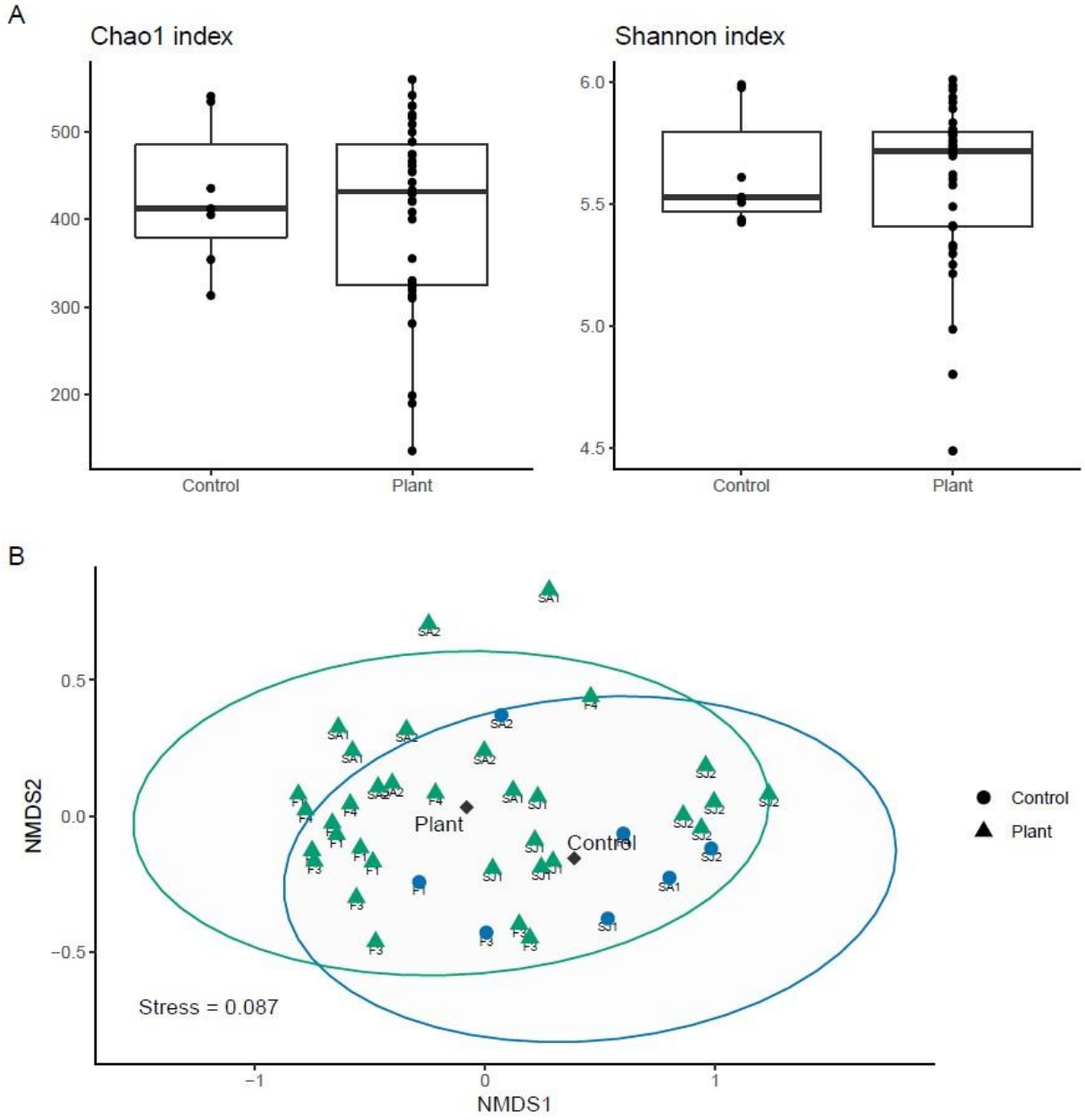

Figure 11. Boxplots (A) illustrating the Shannon diversity index and Chao1 index (richness) of the bacterial communities compared between plant soil samples and control samples. In (B) an NMDS ordination displays the variation in the bacterial composition per samples between the plant soil samples and the control samples.

The relationship was further tested by PERMANOVA which found the variation among the bacterial communities significantly affected by sample type. Between the two groups (plant soil- and control sample), effects from other factors where tested and the best model explaining the variation of the bacterial communities contained. Sample type was found to be the only significant factor, explaining around 7% (Table 1).

Table 1. Assessment of the variation of the bacterial communities in response to sample type, assessed with Permutational multivariate analysis of variance (PERMANOVA).

	Df	***SS***	***F-model***	***R^2***	***p-value***
Sample type	1	0.58	3.08	0.07	**0.006**

Relative and differential abundance in plant soil- and control samples

There were significant differences between control and plant soil samples for relative abundance of both phylum and family taxa with over 1% abundance (Figure 12). The most abundant phyla found in plant soil samples was *Actinobacteria* (30.5%) and *Proteobacteria* (31%), followed by *Acidobacteria* (16.3%). In the control samples I found that among phyla *Actinobacteria* (31.6%) had the highest proportion, followed by *Proteobacteria* (25.8%) and then *Acidobacteria* (16.2%). At family level the most abundant taxa in plant soil samples were *Bradyrhizobiaceae* (6.2%), *Isosphaeraceae* (3.8%) and *Pseudonocardiaceae* (3.2%). In the control samples the families with the highest proportions were *Bradyrhizobiaceae* (5.2%) and *Isosphaeraceae* (4.8%; Figure 12).

Through the DESeq2 analysis of the differential abundance I found that there was a significantly higher abundance of the phylum *Bacteroidetes* ($p<0.001$) in the plant soil samples than in the control samples. On a family level I found that the families *Phyllobacteriaceae, Chitinophagaceae* and *Reyranellaceae* had a significantly higher abundance in the plant soil samples than in the control samples. The only family which was significantly higher in the control samples compared to the plant soil samples was the family *Gemmatacae* (Figure 12; Appendix table 2).

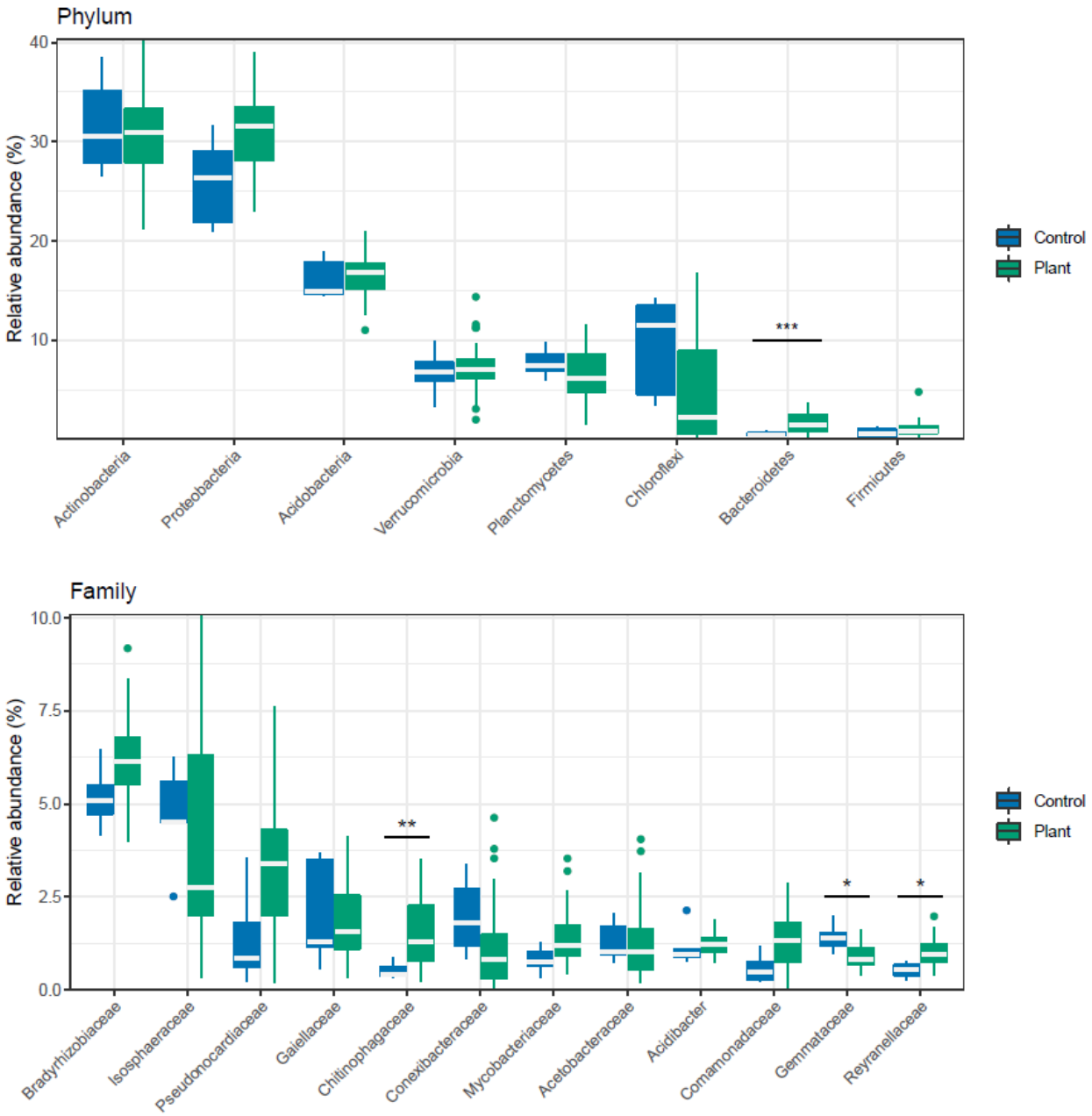

Figure 12. Relative abundance (%) of grouped by plant soil samples and control samples, the plot includes the 8 most abundant bacterial phyla and 12 most abundant bacterial families. Taxa with significant differential abundance are annotated in the plot.

Effects of the elevational gradient in Finse on the bacterial composition in the soil under *Silene acaulis*

Diversity of the bacterial communities along an elevational gradient

After filtering, the dataset used for comparing the bacterial communities along the elevational gradient at Finse contains 1141 unique reads from the processed data and I found 74 unique bacterial families.

The bacterial diversity calculated with Shannon diversity index was not significantly different between the different elevations (Figure 13a). However, when investigating the difference in richness using the Chao1 index I found a significant difference between the mid-elevation and the high elevation (Figure 13a; $p = 0.024$). The results from analyzing beta-diversity using PERMANOVA also indicated a difference in composition between samples from mid-elevation compared to both high and low elevation (Table 2; Figure 13b). But there was not a significant difference between the high and low elevation ($p=0.054$).

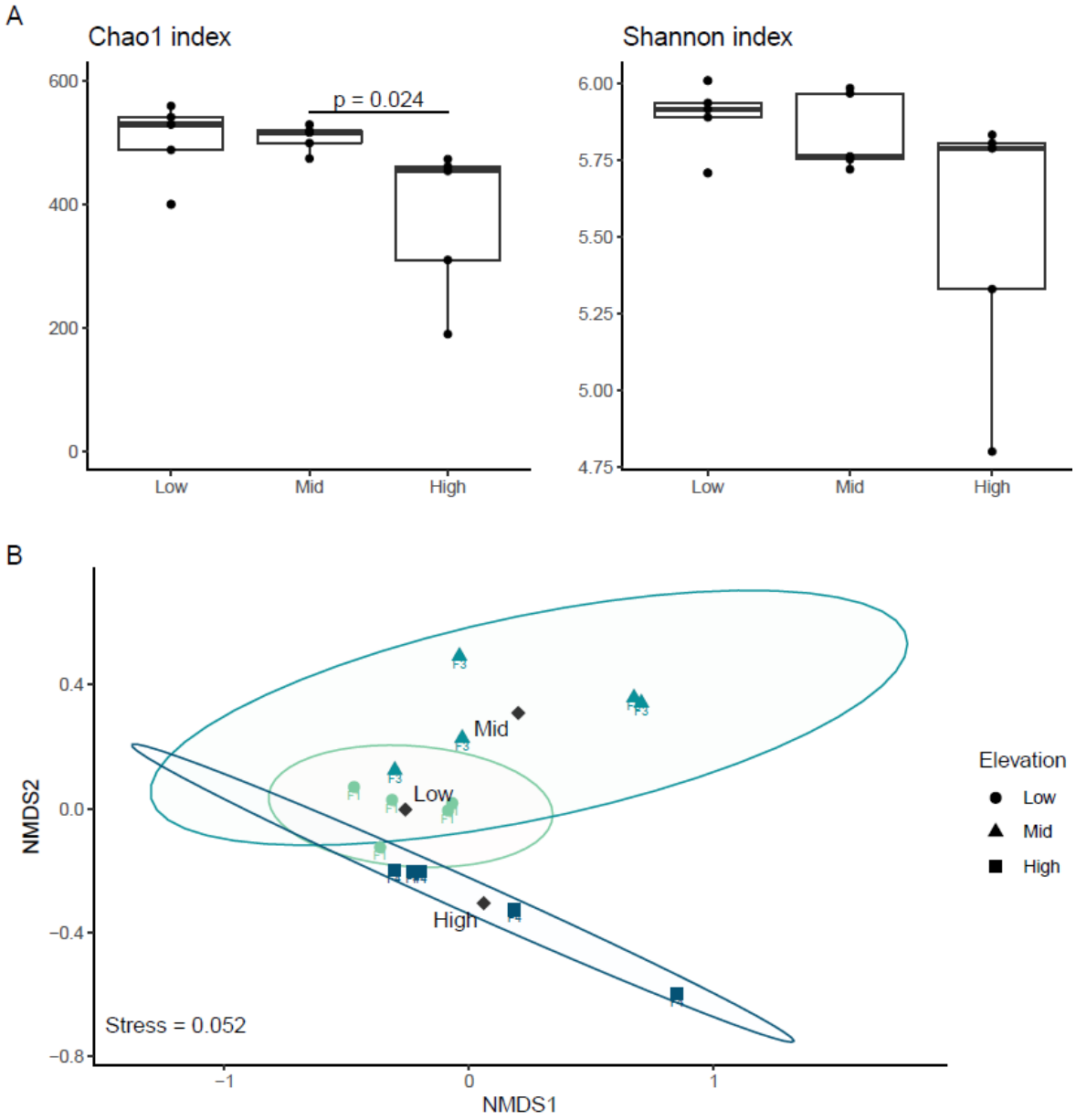

Figure 13. Boxplots (A) illustrating the Shannon diversity index and Chao1 index (richness) of the bacterial communities compared between the different elevations. In (B) a Nonmetric Multidimensional scaling (NMDS) ordination displays the variation in the bacterial composition per samples across the three different elevations at Finse.

Table 2. Pairwise comparison between the three different elevations in Finse calculated with Permutational multivariate analysis of variance (PERMANOVA).

	Overall response			***Mid - Low***		***Low - High***		***Mid - High***	
	Df	**R^2**	**p-value**	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**	**R^2**
Elevation	2	0.29	**0.01**	**0.028**	0.26	0.054	0.18	**0.037**	0.25

The association between the different factors and the variation in the bacterial soil communities was also tested with PERMANOVA. The best model with elevation as the variable of interest was the model with elevation and water content as predictor variables (Table 3).

Table 3. Comparison between models from PERMANOVA results, by Adonis2 for overall model. Estimating effects on the variation of the bacterial community under *Silene acaulis.* Df = degrees of freedom, WC = Mean gravimetrical water content.

Models	***Df***	***R^2***	***p-value***
Elevation	2	0.29	**0.001**
Elevation + WC	3	0.42	**0.001**

The variation can therefore be explained by the different elevations as well as the water content, where elevation explains about 28% of the variation and the water content in the soil around 14% (Table 4).

Table 4. Assessment of the variation of the bacterial communities in response to elevation and water content, assessed with Permutational multivariate analysis of variance (PERMANOVA). WC = Mean gravimetrical water content.

Prediction variables	***Df***	***SS***	***MS***	***R^2***	***P-value***
Elevation	2	0.59	0.30	0.28	**0.003**
WC	1	0.30	0.30	0.14	**0.013**

Relative and differential abundance of the bacterial communities along an elevational gradient

I found that the three phyla *Proteobacteria* (Low = 30.2%, Mid = 32.4%, High = 31.2%), *Actinobacteria* (Low = 32.2%, Mid = 30.85%, High = 30.25%) and *Acidobacteria* (Low = 18.54%, Mid = 14.4%, High = 17.6%) had the highest relative abundance over the three elevations (Figure 14a). From the DESeq analysis of the differential abundance, I found the phylum *Planctomycetes* had a significantly higher abundance in the samples from the mid-elevation compared to both high and low elevation (p-adj < 0.001) and the phylum *Chloroflexi*

showed a significantly higher abundance in the mid-elevation compared to the low elevation (p-adj = 0.0007).

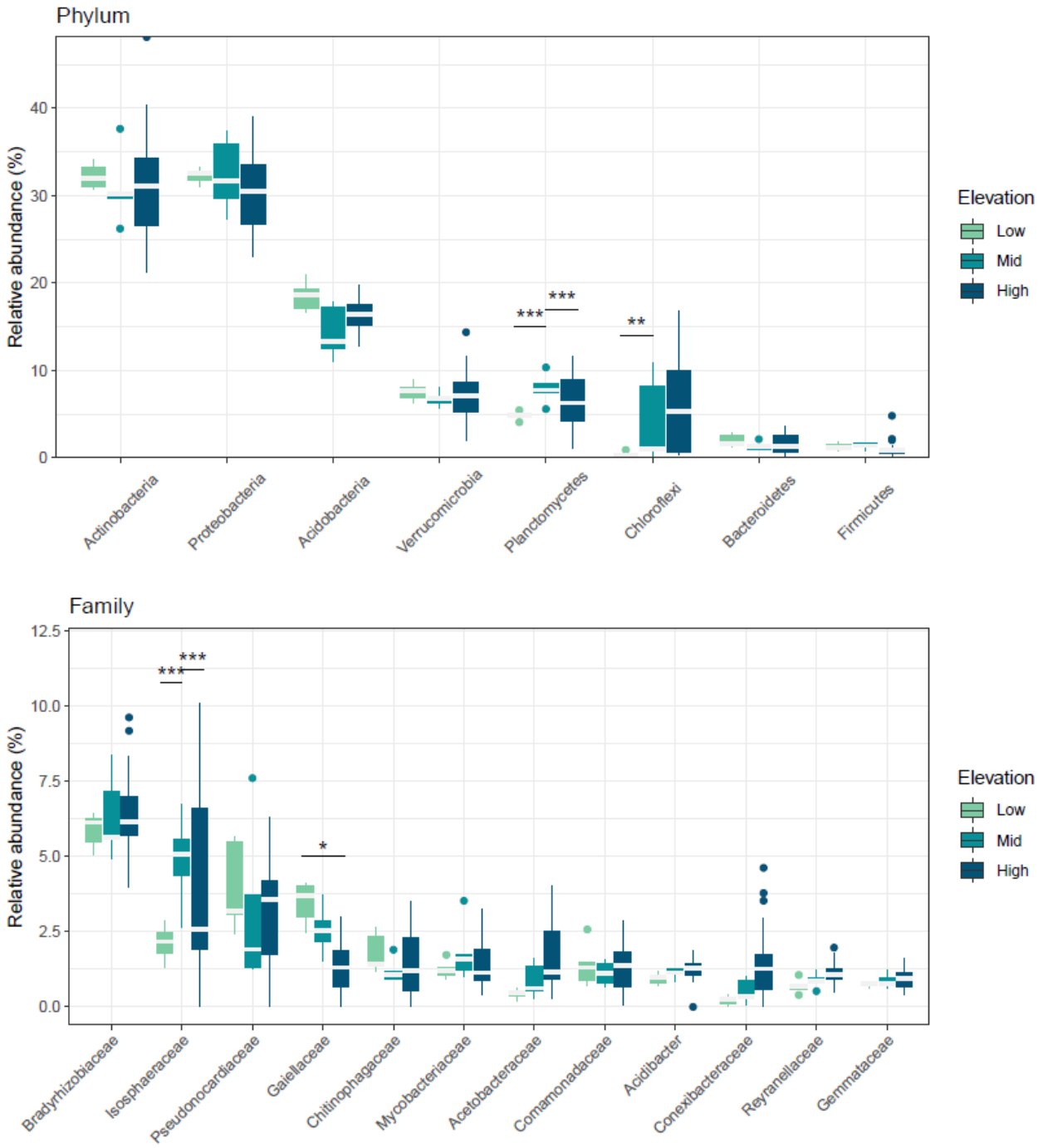

Figure 14. Relative abundance (%) grouped by elevation for the three plant populations at Finse. The plot includes the eight most abundant bacterial phyla and 12 most abundant bacterial families. Taxa with significantly differential abundance are annotated in the plot.

On a family level the most abundant taxa at all elevations were the families *Bradyrhizobiaceae* (Low = 5.9%, Mid = 6.35%, High = 5.7%; Figure 14b)). At the mid elevation the second most abundant family was *Isosphaeraceae* (4.9%), which was significantly higher than both high and low elevation. The second most abundant family for both high and low elevation was the family *Pseudonocardiaceae* (Low = 4%, Mid = 3.17%, High = 4.2%).

Rhodanobacteriaceae was found in significantly higher abundance at the high elevation compared to both low and mid elevations. The family *Intrasporangiaceae* was found in significantly higher abundance at both low and high elevation compared to mid (Figure 14b; Appendix table 3).

Effects of latitudinal gradient on the bacterial composition in the soil under *Silene acaulis*

Diversity of the bacterial communities along a latitudinal gradient

The filtered dataset used for comparing the bacterial communities along the latitudinal gradient consisted of 1267 unique reads from the processed data and 73 unique bacterial families.

The Shannon diversity index showed no significant differences on the gene level among populations from different latitudes (Figure 15a). However, I found significant differences in beta-diversity between populations at low and mid latitudes, and high and mid latitudes but not between low and high latitudes (Figure 15a). In addition, the NMDS ordination reveals differences between mid and low latitudes along the first axis, while the mid and high latitudes are separated along the second axis (Figure 15b).

The overall effect of latitude on the bacterial composition was significant ($p = 0.001$; $R^2=0.33$; Table 5). The main factors best explaining the variation in the bacterial communities were mean soil temperature, soil pH, and mean cushion area (Table 6; $R^2 = 0.55$; p-value = 0.001).

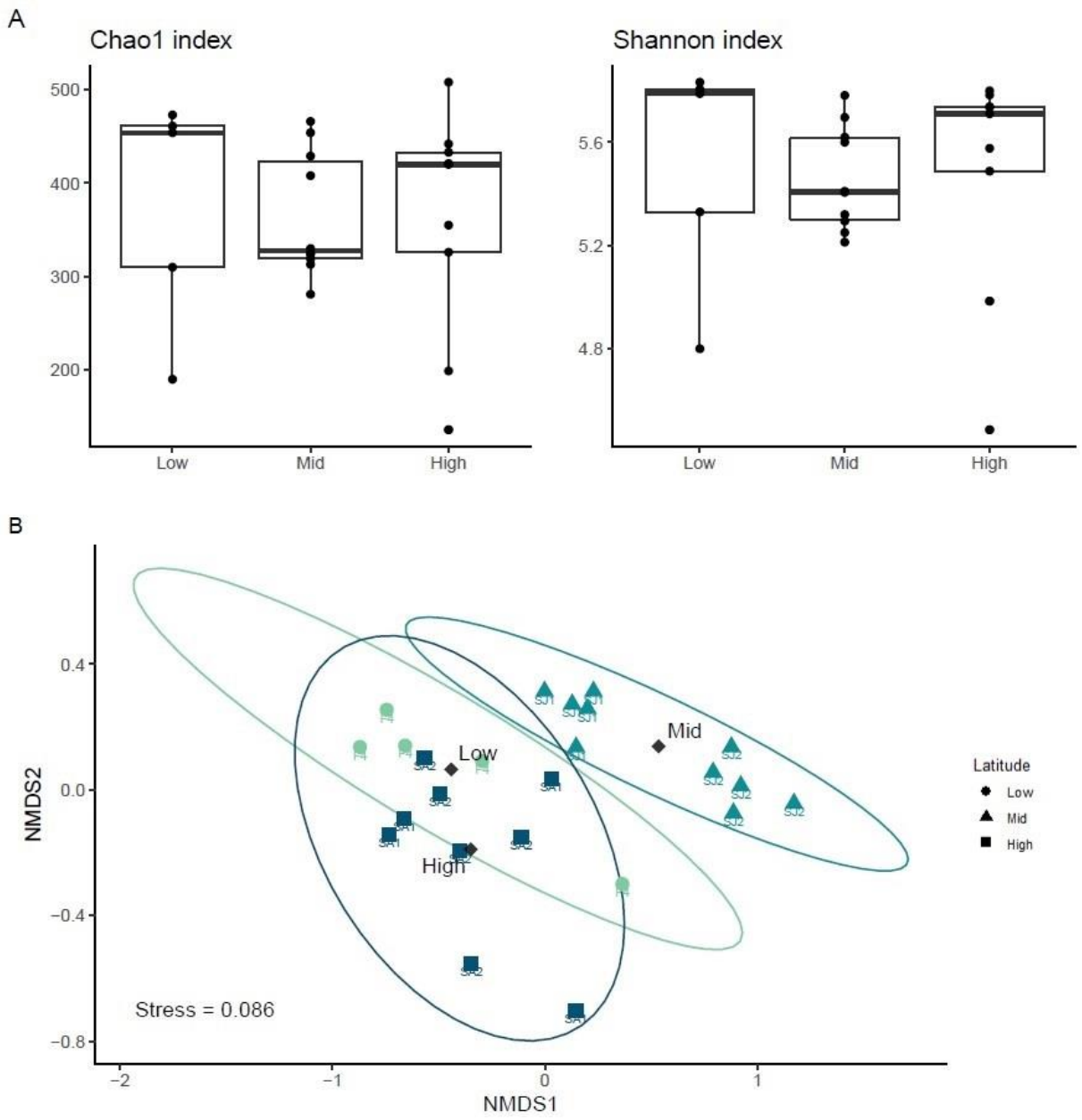

Figure 15. Boxplots (A) illustrating the Shannon diversity index and Chao1 index (richness) of the bacterial communities compared between the different latitudes. In (B) a Nonmetric Multidimensional scaling (NMDS) ordination displays the variation in the bacterial composition per samples across the three different latitudes.

Table 5. PERMANOVA output of the overall response of the variation of the bacterial communities, explained by latitude, and pairwise comparison between the three different latitudes with PERMANOVA.

	Overall response			***Mid - Low***		***Low - High***		***Mid - High***	
	Df	**R^2**	**p-value**	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**	**R^2**
Latitude	2	0.33	**0.001**	**0.002**	0.32	**0.032**	0.14	**0.001**	0.29

Table 6. The stepwise procedure for each added variable, leading to the best fitting model. Output from the PERMANOVA. AC = Average log(cushion area) per site. ST = Mean soil temperature

Models	***Df***	***Sum of Sqs***	***F.Model***	***R^2***	***p-value***
Latitude + ST	3	1.0	5.4	0.45	**0.001**
Latitude + ST + pH	4	2.36	5.33	0.53	**0.001**
Latitude + ST + pH + AC	5	2.7	5.05	0.58	**0.001**

The results from the selected model indicates latitude as the factor explaining the most of the variation with R^2 = 0.33, mean soil temperature explains 11%, mean pH 8% and mean log(cushion area) explains 5% of the variation (Table 7).

Table 7. Assessment of the variation of the bacterial communities in response to latitude, mean soil temperature, mean pH and mean log(cushion area) assessed with Permutational multivariate analysis of variance (PERMANOVA).

Prediction variables	***Df***	***Sum of Sqs***	***F.Model***	***R^2***	***P-value***
Latitude	2	1.51	7.3	0.33	**0.001**
Mean soil temperature	1	0.49	4.8	0.11	**0.002**
Mean pH	1	0.36	3.48	0.08	**0.01**
Mean log(Cushion area)	1	0.24	2.24	0.05	**0.038**

Relative and differential abundance of the bacterial communities along a latitudinal gradient

The three most abundant phyla in all three latitudes were *Proteobacteria* (Low = 31.2%, Mid = 26.8%, High = 34.6%), *Actinobacteria* (Low = 31.2%, Mid = 29.9%, High = 30.3%) and *Acidobacteria* (Low = 17.6%, Mid = 16.6%, High = 15.5%) (Figure 16). I found the phylum *Proteobacteria* was significantly less abundant at the mid latitude compared to the high latitude. The phylum *Chloroflexi* was more abundant at the mid latitude than both high and low latitudes. The phylum *Planctomyctes* was also found to be significantly more abundant in the mid latitude compared to the low latitude. The phyla *Firmicutes* and *Bacteroidetes* had a significantly higher abundance in the high and low latitudes compared to the mid latitude, and *Acidobacteria* had a

significantly lower abundance at the high latitude compared to the mid and low latitude (Figure 16, Appendix table 4).

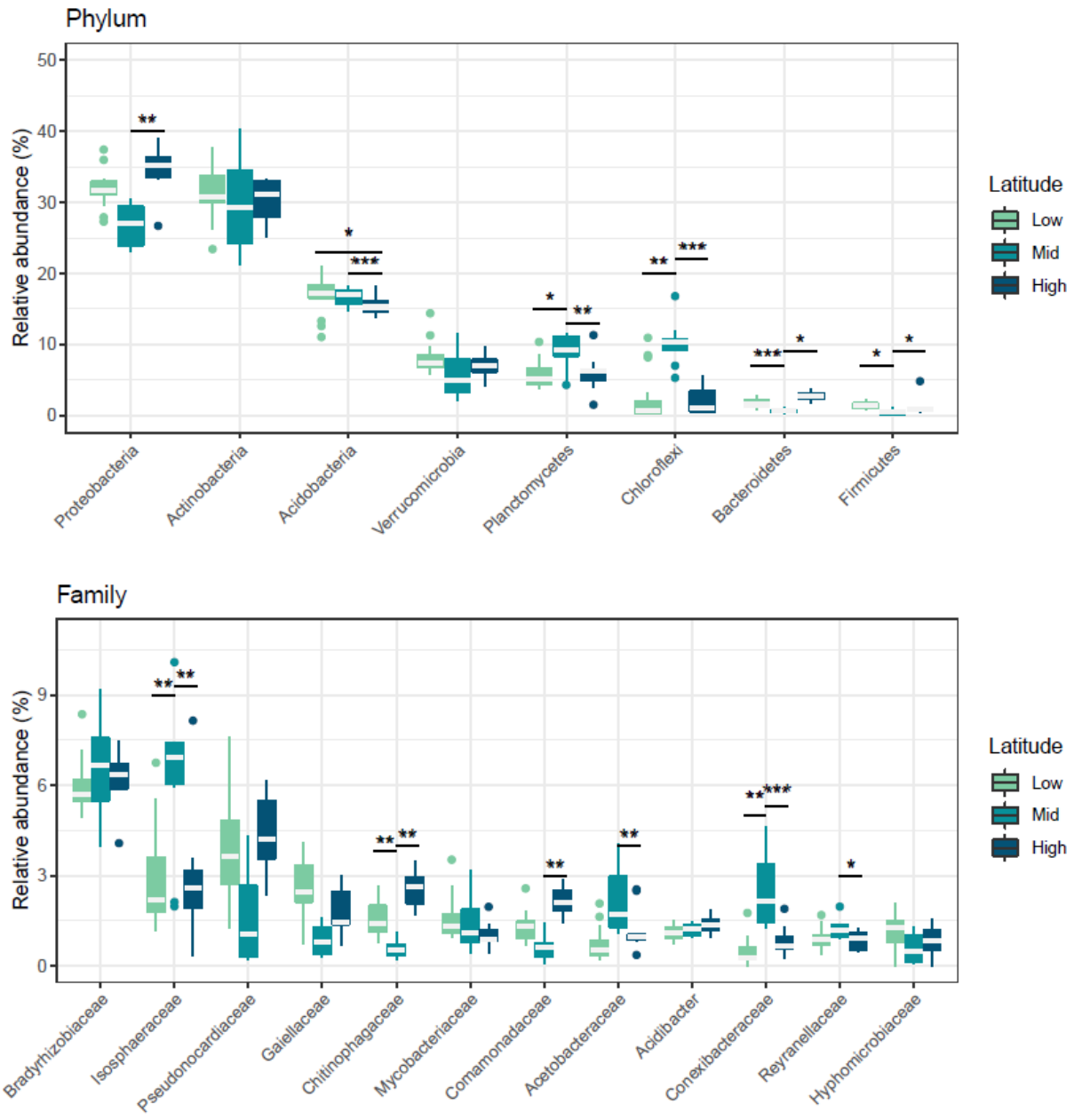

Figure 16. Relative abundance (%) for bacteria at family and phylum level per latitude, showing the 8 most abundant bacterial phyla and the 12 most abundant families. Taxa with significantly differential abundance are annotated in the plot.

At the family level, the most abundant taxon was the family of *Bradyrhizobiaceae* for all three latitudes (Low = 5.4%, Mid= 6.8%, High = 6.2%). For the low and high latitudes the second most abundant family was *Pseudonocardiaceae* (Low = 4%, Mid = 1.2%, High = 4.3%). The family *Isosphaeraceae*, the second most abundant at the mid latitude, was significantly more

abundant at mid latitudes than at low and high (Low = 1.56%, Mid = 6.7%, High = 2.9%, p = 0.001, p = 0.007, respectively). Additionally, *Conexibacteraceae* had significantly greater abundance at mid latitude sites when compared with both high and low latitude sites. Whereas the family *Chitinophagaceae* had a significantly lower abundance at the mid latitude sites compared to the low and high latitude sites (Figure 16; Appendix table 5).

Discussion

Here, I investigated the relationship between the alpine cushion plant *Silene acaulis* and the bacterial communities living in the soil rhizosphere. I found a significant effect of *Silene acaulis* on the composition of the soil bacterial communities when compared to soil bacterial communities found in bare soil. However, there was no significant effect of *S. acaulis* on the diversity or richness of the soil bacterial community when compared to bacterial communities in bare soil. In other words, S. acaulis appears to influence the relative abundance and community structure of soil bacterial communities, but not the overall number of taxa within the soil of the rhizosphere.

I also explored how the composition and diversity of the bacterial communities under *S. acaulis* may change in response to changes in latitude and elevation. I found that the increase in elevation had a negative effect on the richness of soil bacteria communities at Finse. Here the overall number of taxa found in the soil from *S. acaulis* seemed to be lowest at the high elevation compared to the mid and low elevation. However, the soil bacterial composition was only different at the mid elevation site when compared to the high and low elevation sites. Therefore, it seems that while higher elevation reduces the number of soil bacterial taxa under *S. acaulis*, the impact of elevation on soil bacterial communities structure is relatively weak.

Latitude was found to explain 33% of the bacterial composition in the rhizosphere of *S. acaulis.* However, the dissimilarity was highest between the mid latitude compared to both the low and high latitude. Moreover, the difference in latitude had no significant effect on the richness or Shannon diversity of the bacterial soil communities. This means that difference in latitude had no influence on the number of bacterial taxa under *S. acaulis*. Furthermore, as the effect of difference in elevation, the influence from latitude on the bacterial community structure also here appears to be weak.

Effects of *Silene acaulis* on its soil bacterial communities

I found that *Silene acaulis* had a significant influence on the composition of the bacterial communities in the soil under its cushion. The effect of *S. acaulis* explained 7% of the variation ($p = 0.017$) between the samples. Roy et al. (2013) found similar results, where the presence of *S. acaulis* was also found to have an influence on the composition of the bacterial communities.

The most abundant phyla found in the soil under *S. acaulis* were similarly abundant in bare soil (Figure 12). *Actinobacteria* and *Proteobacteria* were the most abundant phyla in both groups, making up over 60% of the relative abundance in the plant soil samples and over 55% in the control samples. The phylum *Proteobacteria* is often found to be the dominating phylum in arctic and alpine soils, thus the high abundance of this phylum was expected. However, compared to previous studies on alpine and arctic soils, I found a higher abundance of *Actinobacteria* than expected (Malard et al., 2018; Marian et al., 2022).

Most species of *Actinobacteria* are saprophytic with several taxa able to degrade chitin, lignin, creatine and pectin, which increase both the availability of nutrients and soil organic carbon in the soil (Brzezinska et al., 2014). Adamczyk et al. (2020) found that an increase in litter in high arctic soils revealed a significant increase of the phyla *Actinobacteria, Proteobacteria* and *Bacteroidetes.* Additionally, they found that litter amended soils decreased the abundance of *Acidobacteria* and *Verrucomicrobia*. They concluded that their results supported the importance of the copiotrophic/oligotrophic life strategy for bacteria in this environment. Thus, it could be speculated that the my study sites are generally richer in soil organic carbon than what is common in alpine and arctic environments, due to the high abundance of *Actinobacteria* found here.

The phylum *Bacteroidetes* was found to have a significantly higher abundance in soil under *S. acaulis* compared to bare soil. *Bacteroidetes* was mainly composed of the family *Chitinophagaceae,* a chitinolytic family (DeLong et al., 2014a) which was also significantly more abundant in *S. acaulis* soil compared to bare soil. The richness and abundance of arthropods is known to be higher under the cushion of *S. acaulis* compared to surrounding vegetation (Luptacik et al., 2021; Molenda et al., 2012). Thus, the higher abundance of *Chitinophagaceae* under *S. acaulis* may be due to a higher quantity of chitinous material from arthropods.

Among the most abundant families found in plant soil were *Bradyrhizobiaceae, Comamonadaceae,* which are characterized as copiotrophs (Francioli et al., 2016; Ogata et al., 2020), and *Pseudonocardiaceae*. Comparing the relative abundance between the plant and the bare soil samples, these families showed a trend toward having a higher relative abundance in the plant soil samples. All of these families are considered to contain beneficial bacterial species for plants, which for example may contribute to nutrient cycling by degrading organic material, biocontrol of plant diseases, and promoting plant growth (DeLong et al., 2014b; Ezeokoli et al., 2020). For example, the bacterial taxa *Comamonas acidovorans,* belonging to the family

Comamonadaceae, has been found to suppress symptoms of the plant disease Summer patch (Thompson et al., 1998). The genus *Nitrobacter*, part of the family *Bradyrhizombium,* is a nitrifying soil bacteria and known for its plant growth promoting properties (Sime-Ngando et al., 2015).

Among the most abundant families found in the bare soil samples were *Isosphaeraceae* and *Gemmataceae,* which are characterized as oligotrophs. They showed a slightly higher relative abundance in control samples than in plant soil. Bonanomi et al. (2016) reported a higher content of soil organic carbon under *S. acaulis* compared to open areas, which was also indicated by the soil characterization done on the samples in this thesis. The trend seen here for the most abundant families under *S. acaulis* and in bare soil, may therefore have been influenced by a difference in soil organic carbon under *S. acaulis* compared to the soil organic carbon in bare soil.

This suggests that *S. acaulis* is either actively or passively recruiting specific taxa that are beneficial for plant growth. Roy et al. (2013) suggested that *S. acaulis* acted as a biotic filter on the beta-diversity of soil bacterial communities because the cushion provides a stable environment that protects soil bacteria from external factors. As well as providing the soil bacterial communities with a higher soil carbon- and nutrient content.

Effects of elevation on the soil bacterial communities under *Silene acaulis*

The composition of the bacterial communities under *S. acaulis* at the mid elevation site was significantly different than the high and low elevation sites. In addition, there was a strong trend towards differences in soil bacterial communities between the high and low elevation sites, although this was not significant ($p=0.054$). I also found that the composition of the bacterial communities was significantly affected by soil water content (Table 4). Interestingly, the soil water content at the highest elevation was significantly greater than the two lower elevations. Greater soil water content is beneficial for both microorganisms and plants because it increases mineralization rates and the transport of nutrients. Taken together, this suggests that changes in soil water content across elevational gradients may be an important driver for the structure of soil bacterial communities under plants. A possible cause for the changes in soil water content may be cushion size. Larger cushions at the high elevation site, which reduces

evapotranspiration, coupled with cooler temperatures (Griffiths et al., 2009), may result in a higher water content in the soils under *S. acaulis* at Finse (Bonanomi et al., 2016).

Changes in community composition may also be driven by changes in richness. I found a decline in richness of the soil bacterial community with an increase in elevation. The highest elevation site at Finse had significantly lower richness than the mid elevation and showed a trend towards lower diversity than the lowest elevation site ($p=0.083$). This pattern is similar to other studies (Bryant et al., 2008; Fierer et al., 2011) which also found declines in bacterial richness and diversity along elevational gradients. This also mirrors the general pattern of a decline in plant richness and diversity with increasing elevation, above the tree line (Grytnes et al., 2006; Korner, 2021). Although I did not estimate plant richness within my sites, other studies at Finse have found decreasing plant diversity and richness with increasing elevation (Asplund et al., 2022).

I found the effects of Silene acaulis on the soil bacterial community to be limited to changes in community composition rather than changes in taxonomic identity. This is consistent with the general theory that the microbial community in the rhizosphere of plants is a subset of the microbial diversity in the area around the plant (Berg & Smalla, 2009; Haldar & Sengupta, 2015; Roy et al., 2013). This further suggests that as the richness of the plant community around *S. acaulis* decreases, so does the richness in the bacterial community in its rhizosphere due to the lower influx of bacterial species. Thus, the soil bacterial communities under plants may be the result of both the abiotic and biotic environment in which individual plants are growing. Given that soil bacterial communities declined in richness and changed in composition across the elevational gradient, we may expect that as plant communities shift upwards due to a warmer climate, there will be an increase in the soil bacterial community richness in the rhizosphere of *S. acaulis.*

Across all sites, the phyla *Actinobacteria*, *Protebacteria* and *Acidobacteria* were dominant (Figure 14). The bacterial communities at the mid elevation had significantly higher abundances of both *Chloroflexi* and *Planctomyctes* than both the low and high elevation sites (Figure 14). In the phylum of *Planctomyctes* I found the family *Isosphaeraceae* had a significantly higher abundance at the mid elevation than the other two sites. This may be explained by differences in the soil character between the mid elevation compared to the low and high elevation sites. The soil under *S. acaulis* at the mid elevation site contained more sand than the other sites and the bare soil at this site was mainly clay. Soil type can have strong effects on the bacterial community composition (Berg & Smalla, 2009; Fierer, 2017). Therefore, it may be that because

the soil at the mid elevation contains more sand, it also contains less soil organic carbon, making it more oligotrophic. This is consistent with the preferred habitats of the phyla *Chloroflexi* and *Planctomycetes*, which are generally considered oligotrophs.

Effects of latitude on the soil bacterial communities under *Silene acaulis*

I investigated how the bacterial communities in the soil under *S. acaulis* was influenced by differences in latitude. There was no significant difference in either richness or diversity between the different latitudes (Figure 15). This suggests that the significant differences in abiotic conditions, such as soil temperature, soil moisture, air temperature, and soil pH (Figure 9) were not strong enough to change the richness and diversity of soil bacteria across all three sites. The most abundant phyla found at all latitudes were *Actinobacteria, Proteobacteria,* and *Acidobacteria* with a relative abundance of approximately 30%, 30% and 16% respectively (Figure 16).

Even though there were no significant differences in richness or diversity, there was a significant difference in soil microbial community composition between all three latitudes (Figure 15). In fact, latitude explained the greatest amount of variance (33%) in the bacterial community composition (Table 7). However, I found a lower similarity between mid latitude compared to high and low latitude sites, indicated in both the NMDS ordination (Figure 15) and the PERMANOVA (Table 5). Moreover, the NMDS ordination indicated that each site at the mid latitude was more similar within each population and less similar compared to each other, even though they were at the same latitude and geographically close (~300m). This is in contrast to the high latitude sites, which showed no separation between the two sites despite being geographically very far apart (~30km).

This suggests that site specific factors may be more influential on the bacterial composition than the latitude itself. For example, vegetation community cover, soil characteristics, or bedrock may be important determinants of community composition, but were not included in the model. Ren et al. (2018) found that pH and plant vegetation had the highest influence on soil microbial communities over a latitudinal gradient. The mean pH at the mid latitude was lower than five (Figure 9), while the high and low latitudes had a pH above five. This slightly more acidic environment may cause a shift in the bacterial communities (Table 7). The lower

pH at the mid latitude sites may be explained by the bedrock, which is gneiss. Gneiss is a felsic type of rock, which may cause the soil to be more acidic and poorly developed (Arnesen et al., 2007). A comparison between the two populations at the mid latitude shows that site SJ2 is the deviating site, with a pH of 4.4. Generally, a pH below 5.5 is considered to be optimal for microbes that are acidophiles, and between pH 5-9 is generally optimal for neutrophiles (DeLong et al., 2013). This may explain the difference in the composition between the two sites at the mid latitude, as well as the difference between the mid latitude compared to the high and low latitude.

Soil temperature was the second most influential factor on the bacterial composition after latitude, explaining 11% of the variation. Among my sites, soil temperature decreased with increasing latitude. As temperature is one of the most important factors driving the bacterial community structure (Fierer, 2017), this could indicate a climatic effect on the composition of the soil bacterial community. However, I only measured the mean temperature throughout the growing season. It may be that temperature during other times of the year, such as winter, affect the community composition as well, but were not included in the model.

When investigating the bacterial taxa at these sites, I found a significantly higher abundance of the phyla *Chloroflexi* and *Planctomycetes*, as well as the family *Isosphaeraceae* at the mid latitude sites. Most species within the family *Isosphaeraceae* have been found to be acid-tolerant, which may explain why I found this family at a higher abundance at this latitude (DeLong et al., 2014a). At the mid latitude there was also a significantly lower abundance of both *Firmicutes* and *Bacteroidetes.* Lauber et al. (2009) found that *Bacteroidetes* along with *Actinobacteria*, decreased in abundance with decreasing pH. Moreover, Zhang et al. (2022) found that the activity of *Firmicutes* decreased with a decrease in pH, which at the same time also decreased the nitrification in the soil. These studies indicates that the relative abundance of these taxa could be influenced by the more acidic soil found at the mid latitude. However, I did not see any decrease in the abundance of *Actinobacteria* at this latitude. Therefore, it may be that the lower abundance of *Firmicutes* and *Bacteroidetes* is due to other factors specific to the mid latitude sites which were outside the scope of this study. Additionally, the soil pH measurements may have been affected by the long transport time and the freezing and thawing process.

Conclusion

Silene acaulis was found to have a significant effect on the soil bacterial community in its soil rhizosphere. The better developed soils found under the *S. acaulis* is likely a contributing factor to this difference, as well as protection against extreme changes in temperature and soil moisture. There also appears to be a trend towards a higher abundance of plant beneficial bacterial taxa under the cushion, which may indicate that the plant is influencing the structure of the bacterial community.

Soil bacterial richness under *S. acaulis* decreased with increasing elevation, which is in accordance with previous studies and mirrors the change in diversity found in plant communities along elevational gradients. This suggests that the richness of the soil bacterial communities characterized here are affected by both *S. acaulis* and the total vegetation community. It also suggests that the richness of the soil bacterial communities at high elevations may increase in diversity and richness with a warmer climate as new plant species migrate up to higher elevations.

Along the latitudinal gradient, *S. acaulis* may have a converging effect as we see a more similar composition between high and low latitude compared to the mid latitude. In addition, pH and other site specific factors, such as soil nutrient content, soil organic carbon content, best explain the difference in the soil bacterial composition between the mid latitude sites and the high and low latitude sites.

Taken together, my results suggest that soil bacterial communities are a reflection of a complex suite of site specific factors. Plant identity, plant community diversity, as well as temperature, soil moisture, soil carbon content, and soil pH are important drivers of soil bacterial community composition. However, further research is needed to better separate these factors from each other in order to understand whether a hierarchy among the different factors exists.

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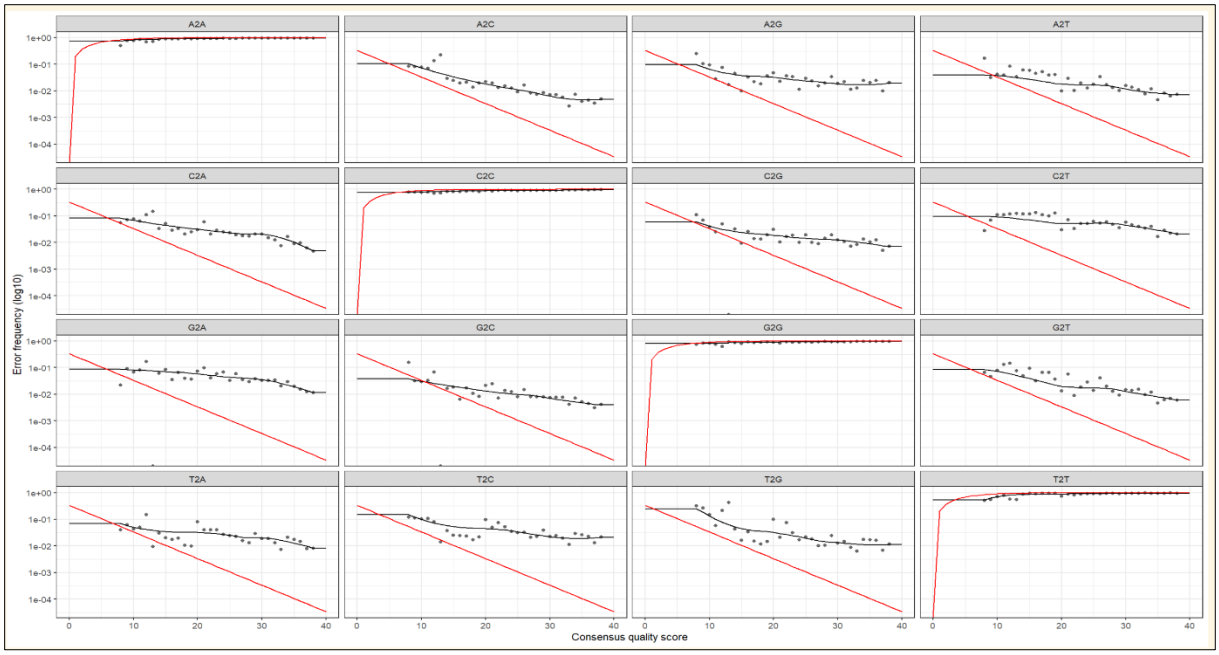
Appendix

Appendix table 1. Relative abundance for, plant soil samples, control samples, low, mid and high latitude and elevation. Includes all families detected in data.

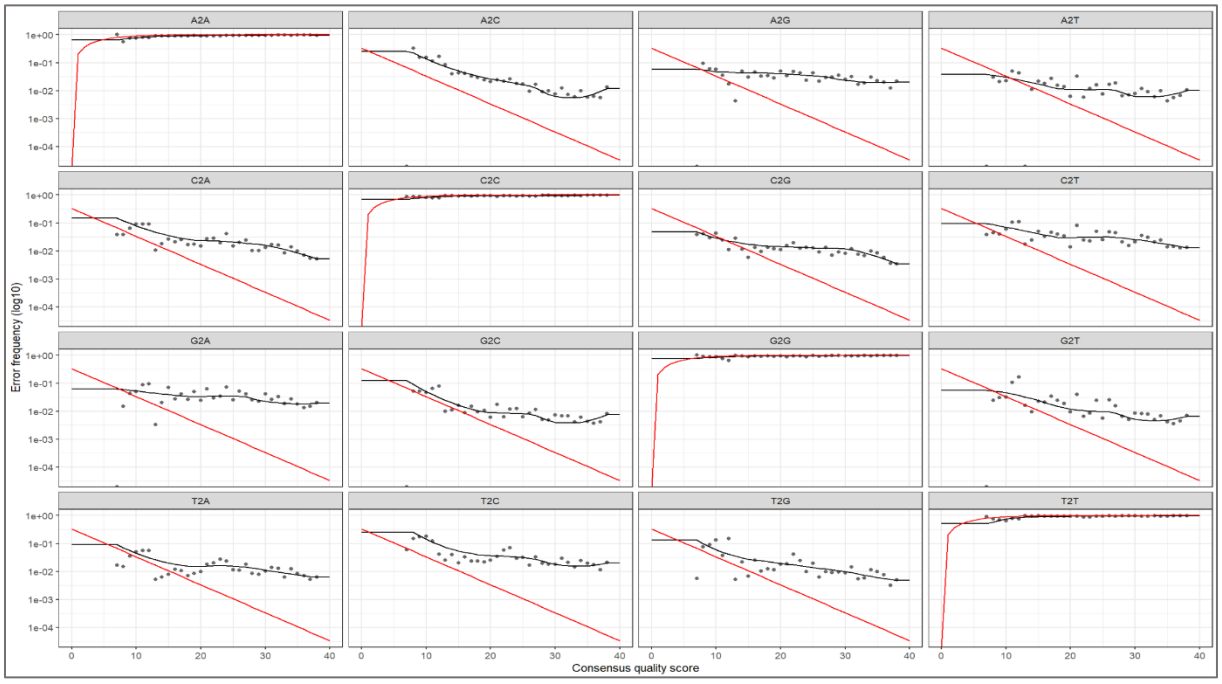
Relative abundance (%) of all families in study								
Phylum	Family	Plant	Control	Low elevation	Mid elevation	High elevation/ Low latitude	Mid latitude	High latitude
Acidobacteria	*Blastocatellaceae*	0.059	0.000	0.116	0.047	0.102	0.000	0.077
Actinobacteria	*Pseudonocardiaceae*	3.308	1.348	3.966	3.166	4.232	1.660	4.338
Actinobacteria	*Gaiellaceae*	1.856	2.118	3.463	2.560	1.660	0.859	1.788
Actinobacteria	*Conexibacteraceae*	1.161	1.969	0.211	0.511	0.695	2.482	0.840
Actinobacteria	*Thermomonosporaceae*	0.546	1.456	0.018	0.620	0.109	1.224	0.288
Actinobacteria	*Mycobacteriaceae*	1.384	0.815	1.247	1.819	1.720	1.380	1.034
Actinobacteria	*Micromonosporaceae*	0.722	0.198	1.014	0.805	0.623	0.160	1.194
Actinobacteria	*Intrasporangiaceae*	0.703	0.199	1.547	0.372	1.582	0.076	0.628
Actinobacteria	*Solirubrobacteraceae*	0.694	0.182	1.399	0.773	1.228	0.022	0.709
Actinobacteria	*Microbacteriaceae*	0.374	0.064	0.638	0.411	0.206	0.007	0.708
Actinobacteria	*Iamiaceae*	0.372	0.150	0.751	0.751	0.244	0.068	0.361
Actinobacteria	*Nakamurellaceae*	0.354	0.095	0.583	0.503	0.223	0.023	0.584
Actinobacteria	*Baekduiaceae*	0.293	0.321	0.334	0.184	0.313	0.296	0.317
Actinobacteria	*Kineosporiaceae*	0.280	0.089	0.363	0.342	0.330	0.028	0.454
Actinobacteria	*Acidimicrobiaceae*	0.082	0.209	0.016	0.027	0.000	0.250	0.009
Actinobacteria	*Frankiaceae*	0.209	0.192	0.188	0.318	0.070	0.104	0.355
Actinobacteria	*Nocardioidaceae*	0.183	0.072	0.409	0.220	0.136	0.008	0.258
Actinobacteria	*Cellulomonadaceae*	0.134	0.009	0.194	0.254	0.046	0.051	0.174
Actinobacteria	*Ilumatobacteraceae*	0.098	0.051	0.225	0.190	0.079	0.000	0.095
Actinobacteria	*Streptomycetaceae*	0.071	0.028	0.116	0.108	0.000	0.020	0.124
Actinobacteria	*Micrococcaceae*	0.045	0.069	0.090	0.098	0.030	0.005	0.045
Actinobacteria	*Acidothermaceae*	0.064	0.029	0.000	0.014	0.000	0.124	0.096
Actinobacteria	*Streptosporangiaceae*	0.019	0.000	0.012	0.000	0.121	0.000	0.000
Actinobacteria	*Demequinaceae*	0.014	0.000	0.068	0.022	0.000	0.000	0.004
Actinobacteria	*Nocardiaceae*	0.013	0.000	0.032	0.008	0.000	0.000	0.026
Armatimonadetes	*Chthonomonadaceae*	0.113	0.089	0.096	0.067	0.061	0.125	0.165
Bacteroidetes	*Chitinophagaceae*	1.523	0.503	1.795	1.200	1.636	0.565	2.552
Bacteroidetes	*Fulvivirgaceae*	0.107	0.042	0.171	0.130	0.149	0.036	0.115
Chloroflexi	*Dictyobacteraceae*	0.479	0.879	0.031	0.035	0.097	1.249	0.329
Cyanobacteria/Chloroplast	*Streptophyta*	0.074	0.054	0.073	0.110	0.088	0.047	0.077
Firmicutes	*Clostridiaceae 1*	0.776	0.431	0.816	0.819	1.141	0.341	1.009
Firmicutes	*Peptococcaceae 1*	0.052	0.065	0.111	0.126	0.020	0.050	0.000
Firmicutes	*Planococcaceae*	0.045	0.065	0.017	0.060	0.065	0.007	0.084
Firmicutes	*Paenibacillaceae 1*	0.053	0.009	0.087	0.028	0.134	0.014	0.045
Firmicutes	*Bacillaceae 1*	0.031	0.000	0.169	0.000	0.025	0.008	0.000
Firmicutes	*Sporomusaceae*	0.012	0.000	0.000	0.081	0.000	0.000	0.000

Gemmatimonadetes	*Gemmatimonadaceae*	0.324	0.726	0.644	0.366	0.368	0.142	0.301
Nitrospirae	*Nitrospiraceae*	0.069	0.066	0.132	0.137	0.154	0.024	0.000

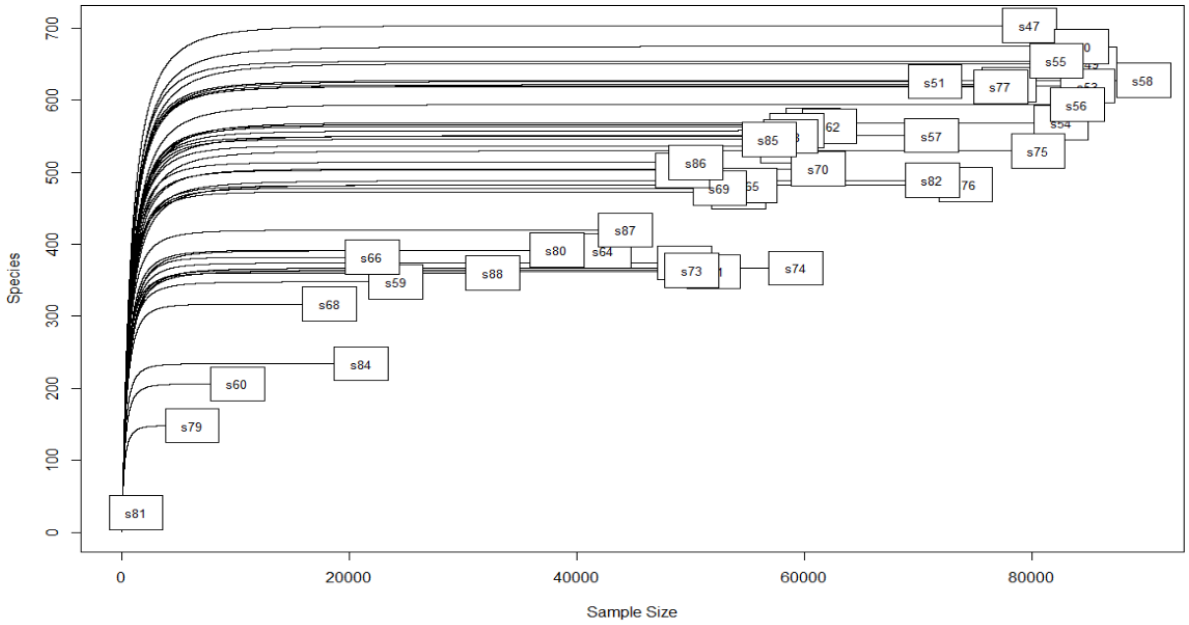
Planctomycetes	*Isosphaeraceae*	*3.904*	*4.782*	*2.119*	*4.885*	*1.714*	*6.264*	*2.944*
Planctomycetes	Gemmataceae	0.912	1.394	0.753	0.863	0.791	1.013	0.980
Planctomycetes	Planctomycetaceae	0.250	0.238	0.164	0.279	0.120	0.301	0.297
Planctomycetes	Thermoguttaceae	0.201	0.146	0.232	0.146	0.165	0.070	0.381
Planctomycetes	Lacipirellulaceae	0.169	0.036	0.297	0.205	0.297	0.010	0.184
Proteobacteria	Bradyrhizobiaceae	6.239	5.162	5.878	6.347	5.706	6.657	6.210
Proteobacteria	Comamonadaceae	1.327	0.559	1.395	1.116	1.369	0.634	2.152
Proteobacteria	Acetobacteraceae	1.283	1.297	0.443	0.884	0.833	2.183	1.223
Proteobacteria	Acidibacter	1.218	1.110	0.952	1.135	1.251	1.212	1.401
Proteobacteria	Reyranellaceae	0.988	0.522	0.686	0.884	1.188	1.230	0.832
Proteobacteria	Hyphomicrobiaceae	0.918	0.630	1.323	1.333	0.900	0.573	0.857
Proteobacteria	Polyangiaceae	0.814	0.352	0.900	0.557	0.682	0.448	1.390
Proteobacteria	Enterobacteriaceae	0.678	0.264	0.000	0.340	0.000	0.181	2.170
Proteobacteria	Roseiarcaceae	0.451	0.306	0.025	0.253	0.356	0.851	0.405
Proteobacteria	Phyllobacteriaceae	0.307	0.016	0.389	0.160	0.495	0.033	0.543
Proteobacteria	Steroidobacteraceae	0.281	0.226	0.207	0.079	0.301	0.345	0.352
Proteobacteria	Oxalobacteraceae	0.220	0.220	0.287	0.160	0.536	0.132	0.137
Proteobacteria	Geobacteraceae	0.188	0.173	0.575	0.554	0.032	0.059	0.000
Proteobacteria	Rhodanobacteraceae	0.186	0.045	0.147	0.162	0.341	0.073	0.260
Proteobacteria	Burkholderiaceae	0.159	0.126	0.074	0.060	0.099	0.135	0.322
Proteobacteria	Rhizobiaceae	0.156	0.035	0.133	0.065	0.187	0.062	0.307
Proteobacteria	Caulobacteraceae	0.056	0.141	0.067	0.024	0.022	0.133	0.000
Proteobacteria	Methylocystaceae	0.063	0.140	0.000	0.135	0.000	0.125	0.024
Proteobacteria	Labilitrichaceae	0.135	0.094	0.115	0.184	0.249	0.081	0.114
Proteobacteria	Pseudomonadaceae	0.119	0.040	0.162	0.064	0.167	0.043	0.182
Proteobacteria	Beijerinckiaceae	0.015	0.110	0.000	0.006	0.000	0.049	0.000
Proteobacteria	Xanthobacteraceae	0.097	0.023	0.112	0.110	0.049	0.007	0.206
Proteobacteria	Anaeromyxobacteraceae	0.047	0.095	0.181	0.098	0.000	0.020	0.000
Proteobacteria	Devosiaceae	0.092	0.000	0.140	0.065	0.131	0.030	0.128
Proteobacteria	Sphingomonadaceae	0.054	0.000	0.079	0.036	0.043	0.007	0.109
Proteobacteria	Micropepsaceae	0.051	0.028	0.128	0.015	0.068	0.030	0.043
Proteobacteria	Azospirillaceae	0.040	0.030	0.104	0.088	0.055	0.000	0.015
Proteobacteria	Geminicoccaceae	0.034	0.007	0.086	0.060	0.020	0.000	0.036
Proteobacteria	Rhodospirillaceae	0.026	0.000	0.014	0.029	0.051	0.000	0.046
Proteobacteria	Hyphomonadaceae	0.023	0.000	0.064	0.014	0.081	0.000	0.000
Verrucomicrobia	Opitutaceae	0.117	0.088	0.115	0.100	0.169	0.053	0.168



Appendix figure 1. Error model forward reads, DADA2 pipeline.



Appendix figure 2. Error model reverse reads, DADA2 pipeline.



Appendix figure 3. Rarefaction curve, for all samples. Including outlier sample 35 (sample s81 in plot).

Appendix table 2. Differential abundance on family level from DESeq analysis, indicating the Log2 Fold Change of the bacterial families between plant soil- and control sample bacterial communities.. Plant vs Control. Table is including taxa with mean total abundance > 100.

Log2FoldChange	lfcSE	padj	Family
Control vs Plant			
0.716	0.23	0.03	*Reyranellaceae*
1.44	0.39	0.005	*Chitinophagaceae*
-0.516	0.18	0.04	*Gemmataceae*
3.82	1.28	0.04	*Phyllobacteriaceae*

Appendix table 3. Differential abundance from DESeq analysis. indicating the Log2 Fold Change of the bacterial families between bacterial communities sampled from population at different elevations at Finse. Significance level p-adjusted < 0.05. Table is including taxa with mean total abundance > 100.

Log2 Fold Change	Lfc SE	p-adjusted	Family
Low vs Mid			
1.34	0.31	0.0005	*Isosphaeraceae*
-2.004	0.57	0.006	*Intrasporangiaceae*

24.43	3.45	0.000	*Enterobacteriaceae*
High vs Mid			
1.79	0.31	0.000	*Isosphaeraceae*
-1.89	0.56	0.01	*Intrasporangiaceae*
1.95	0.62	0.02	*Iamiaceae*
Low vs High			
-1.23	0.40	0.04	*Gaiellaceae*
-4.15	0.79	0.00	*Geobacteraceae*
1.26	0.35	0.01	*Rhodanobacteraceae*

Appendix table 4. Differential abundance on phylum level from DESeq analysis. Log2 Fold Change in pairwise comparison between bacterial communities on the phylum level. Latitudinal gradient. Significance level p-adjusted < 0.05. Table is including taxa with mean total abundance > 100.

Log2 Fold Change	**Lfc SE**	**p-adjusted**	**Phylum**
Mid vs Low			
-1.02	0.339	0.032	*Planctomycetes*
-2.12	0.572	0.004	*Chloroflexi*
1.27	0.457	0.016	*Bacteroidetes*
1.43	0.583	0.032	*Firmicutes*
High vs Mid			
-0.23	0.103	0.007	*Proteobacteria*
0.29	0.06	0.000	*Acidobacteria*
0.62	0.23	0.007	*Planctomycetes*
2.011	0.468	0.000	*Chloroflexi*
-2.080	0.374	0.000	*Bacteroidetes*
-1.410	0.477	0.014	*Firmicutes*
High vs Low			
0.20	0.07	0.04	*Acidobacteria*

Appendix table 5. Differential abundance on family level from DESeq analysis. Latitudinal gradient. Significance level p-adjusted < 0.05. Table is including taxa with mean total abundance > 100.

Log2 Fold Change	Lfc SE	p-adjusted	Family
Mid vs low			
-1.846	0.488	0.001	*Isosphaeraceae*
1.381	0.393	0.003	*Chitinophagaceae*
-1.393	0.500	0.028	*Acetobacteraceae*
-1.911	0.492	0.001	*Conexibacteraceae*
-23.192	2.711	0.000	*Enterobacteriaceae*
-2.760	0.682	0.001	*Dictyobacteraceae*
-3.501	1.249	0.028	*Thermomonosporaceae*
6.169	1.696	0.002	*Solirubrobacteraceae*
4.476	1.071	0.000	*Intrasporangiaceae*
High vs Mid			
1.305	0.409	0.007	*Isosphaeraceae*
-1.910	0.330	0.000	*Chitinophagaceae*
1.810	0.412	0.000	*Conexibacteraceae*
-1.606	0.419	0.001	*Comamonadaceae*
0.621	0.228	0.025	*Reyranellaceae*
-1.447	0.370	0.001	*Polyangiaceae*
-3.035	1.039	0.014	*Micromonosporaceae*
2.174	0.570	0.001	*Dictyobacteraceae*
-5.112	1.426	0.002	*Solirubrobacteraceae*
-3.002	0.900	0.004	*Intrasporangiaceae*
-4.935	1.409	0.003	*Microbacteriaceae*
-3.957	1.238	0.007	*Phyllobacteriaceae*
High vs Low			
-26.672	2.75	0.000	*Enterobacteriaceae*

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