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ARCTIC MYCORRHIZAL INTERACTIONS UNDER CLIMATE CHANGE

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Abstract

The Arctic is experiencing climate change at an extreme rate, affecting both the rates of plant growth and nutrient cycling. Due to the changes in temperature, but also in nutrient cycling, with increased nutrient mineralization and decreasing P:N ratio (phosphorus, P and nitrogen, N), the arbuscular mycorrhizal fungi (AM) that are prevalent in warmer soils have shown the potential to increase in the otherwise unfavorable Arctic environment. Additionally, graminoids that are commonly associated with AM, are increasing in abundance in response to climate change. Despite this, the association between graminoids and AM in the Arctic has rarely been the focus of research, and how it is affected by increasing temperatures is not well understood. This study used an altitudinal gradient in subarctic Scandinavia as a proxy for climate change to show a potential positive relationship between climate change and AM association in the Arctic graminoid *Poa alpina*. A likely positive relationship was found between AM association and temperature, and a potential, although uncertain, negative relationship between soil P and AM association. While no significant relationship was found between tissue P and either temperature, soil P or AM association, a negative relationship was found between tissue N and AM association. The likely relationship found between temperature and AM association is similar to that found in the forb–AM association and contributes to the scare knowledge about the graminoid–AM interaction, while the tissue nutrient analyses indicated that AM association could be most beneficial for the most nutrient limited plants. These trends indicate that their AM association has the potential to improve the fitness of Arctic graminoids in a warmer climate, and if future research confirms these trends, it would further our understanding of why certain Arctic plants benefit from climate change.

Key words: climate change, Arctic ecology, arbuscular mycorrhiza, graminoids, plant ecology, soil ecology, carbon cycle.

Abstract

I Arktis pågår klimatförändringar i en extrem hastighet, vilket påverkar både takten av näringscykeln och av växters tillväxt. På grund av dom stigande temperaturerna, men också den ökade takten av näringscykeln som innebär ökad halten oorganiska näringsämnen i marken, men också markens P:N kvot (fosfor, P och kväve, N), har dom arbuskolära mycorrhiza svamparna (AM) som är vanliga i varmare klimat visat potentialen att spridas i Arktis. Utöver detta har graminoider, som ofta samarbetar med AM, blivit allt vanligare i Arktis som en följd av klimatförändringarna. Ändå har samarbetet mellan graminoider och AM sällan varit i forskningens fokus, och hur samarbetet kommer att påverkas av stigande temperaturer är till stor del okänt. Denna studie använde en höjdgradient i norra Skandinavien för att påvisa ett möjligt positivt förhållande mellan klimatförändringar och samarbetet mellan AM och den arktiska graminoiden *Poa alpina*. Studien fann ett troligt positivt förhållande mellan temperatur och AM association och ett potentiellt, fastän osäkert, negativt förhållande mellan mark P och AM association. Medan inget signifikant förhållande fanns mellan vävnads P och temperatur, mark P eller AM association, fanns ett negativt förhållande mellan vävnads N och AM association. Det troliga förhållandet mellan temperatur och AM association är likt det som har funnits i forb–AM associationen, och bidrar till den lilla kunskap som finns om graminoid–AM associationen, medan vävnadsnäringshaltanalysen visar att AM samarbetet skulle kunna vara mest värdefullt för dom växter som har minst näringstillgång. Dessa förhållanden indikerar att deras AM association har potentialen att förbättra arktiska graminoiders konkurrenskraft i ett framtida varmare klimat, och om fortsatt forskning stödjer dessa förhållanden skulle det öka

vetenskapens förstående om varför vissa arktiska växter gynnas av klimatförändringar mer än andra.

Scientific introduction

The Arctic is experiencing warming at three times the rate of the global average (IPCC, 2021). This rapid change will affect many of the processes behind growth (i.e. the capture of atmospheric carbon in plant biomass) and decomposition (i.e. the release of organically bound carbon). As the Arctic stores up to half of the earths terrestrial below ground carbon, understanding how the processes of growth and decomposition will change is important if climate models are to reliably predict future climate change (Shaver et al., 2000; Tarnocai et al., 2009). A facet of this that is still relatively poorly understood, is how some types of plant–fungi interactions will respond to increasing temperatures and changes in the nutrient cycle and how this could influence the carbon cycle in Arctic ecosystem.

The effect of climate change on Arctic below ground processes

Increasing temperatures generally increase the activity of soil microbes and subsequently litter decomposition (Johannes H. C. Cornelissen et al., 2007; Lu et al., 2013), causing an increased loss of soil carbon to the atmosphere (S. E. Hobbie, 1996). The higher rates of decomposition also increase the content of mineralized soil nutrients (i.e., nutrients not bound in organic matter) in the soil (Schmidt et al., 1999; Schmidt et al., 2002). This should benefit arctic plant growth as plants become less nutrient limited (Chapin & Shaver, 1985). However, it has also been shown that the response of decomposition to climate change is not solely dependent on temperature, but also on soil moisture (e.g. Christiansen et al., 2017) and vegetation composition (Berg, 2018; Sarah E. Hobbie et al., 2012) resulting in varying responses to climate change between microclimates and vegetation-types.

The increasing availability of mineralized nutrients in the Arctic will, however, not benefit all plants equally. Most plants associate with fungal partners and provide the fungi with sugars in exchange for soil nutrients. However, these mycorrhizal fungi vary in their abilities, and different fungal partners are adapted to different environments. Plants associated with ericoid mycorrhizal fungi (ERM), ectomycorrhizal fungi (EM), or dark septate root endophytic fungi (DSE) can utilize organically bound nutrients due to their fungal symbionts. Ericoid mycorrhiza (Basidiomycetous and Ascomycetous fungi globally associated with ~4000 species of heath) and ectomycorrhiza (also Basidiomycetous and Ascomycetous fungi globally associated with ~6000 species of mainly shrubs and trees) have the saprophytic abilities to decompose organic matter and supply its nutrients to their plant hosts (Read & Perez-Moreno, 2003; van der Heijden et al., 2015). Additionally, plants associated with dark septate endophytes have been shown to have increased uptake of P (Haselwandter & Read, 1982), and organically bound N (Giesemann et al., 2020; Hill et al., 2019; Newsham, 2011). DSE are also endophytic Ascomycetous fungi that are relatively common in polar regions (Newsham et al., 2009). They have however been shown to be temperature limited (A. L. Ruotsalainen & Kytöviita, 2004) and partially conflicting results showing DSE to be beneficial, neutral or harmful root endophytes leave their role in arctic soil ecology relatively poorly understood (Newsham, 2011).

Unlike plants associated with ERM, EM or DSE, Arctic plants associated with arbuscular mycorrhizal fungi (AM), which are Glomeromycetous fungi that largely lacks saprophytic abilities (Read & Perez-Moreno, 2003; Schüßler et al., 2001), are only supplied mineralized nutrients by their associated fungi. Worldwide more than 80% of terrestrial plant species

(~200 000) form mutualistic relationships with AM (Varma et al., 2017). However, results from multiple studies have shown AM associated plants to be comparatively uncommon in the Arctic (Bledsoe et al., 1990; Dalpé & Aiken, 1998; Kohn & Stasovski, 1990; Väre et al., 1992). This has traditionally been believed to be due to the unfavorable soil conditions in colder environments, with low microbial activity, low propensity for nitrification, low pH and a relatively high P:N ratio (Read, 1991; Read & Perez-Moreno, 2003) decreasing the benefit of AM symbiosis for plant hosts. However, even if soil conditions are disregarded in experimental studies, the benefits of AM symbiosis decrease in colder temperatures (A. L. Ruotsalainen & Kytöviita, 2004). Therefore, there are likely physiological limitations of AM in cold environments. For instance, AM are incapable of P absorption at 0 ˚C (Wang et al., 2002), the supply of N to the plant host decreases in colder temperatures (A. L. Ruotsalainen & Kytöviita, 2004) and the germination of AM spores in temperatures below +10 ˚C is low, or even absent (Kytoviita, 2005). In contrast, EM have been shown to grow at 2 ˚C and remain active at 0 ˚C (Kytoviita, 2005). Because of the differences in mycorrhizal nutrient uptake and temperature limitation the plant hosts response to climate change is likely dependent on whether they are associated with ERM, EM or AM.

Consequently, potential benefits of AM association for a host plant are ultimately determined by the value of the AM acquired nutrients for the host plant carbon acquisition. Therefore, if the additional carbon acquisition generated by AM association (e.g., due to less energy being allocated to root growth and more to shoot growth) is greater than the carbon cost of AM association (i.e., the sugars transported to the fungi in exchange for nutrients), the symbiosis is beneficial for the plant host. Other potential secondary plant costs/benefits, such as improved water uptake (Püschel et al., 2020) and pathogen resistance (e.g. Wehner et al., 2010), could also be included in this assumption. However, if the Arctic environmental conditions limit the efficiency of AM and they require more carbon to supply the plant with nutrients, the association becomes less favorable.

Due to the close relationship between altitude and temperature (i.e., lower temperatures at higher altitudes), altitudinal temperature gradients are a useful proxy for understanding the long-term effects of climate change. Within the Arctic, some studies have indicated that the extent of AM colonization decreases with increasing altitude (Read & Haselwandter, 1981; Vare et al., 1997) while others have found no effect of altitude (A. L. Ruotsalainen et al., 2004). Additionally, of research studying the plant–AM interaction over altitudinal gradients, a majority has been done on forb species (i.e. non-graminoid herbaceous plants), and the knowledge about the effect of temperature on the graminoid (e.g. grasses and sedges)–AM interaction is generally lacking. Many arctic graminoids have been shown to host AM association (e.g. Allen et al., 1987; Dalpé & Aiken, 1998; Kauppinen et al., 2013), and in a 20 year warming experiment Rudgers et al. (2014) found that the AM colonization of some graminoid species increased in response to warmer temperatures. However, the effect depended on environmental conditions and for most species no significant effect was detected. Interestingly, substantial AM colonization was found in both dry and mesic soils, indicating that the plant–AM association is viable in the dry soils generally associated with lower rates of decomposition and nutrient turnover (Christiansen et al., 2017).

The effect of climate change on Arctic plant communities

In addition to the changes in decomposition and nutrient cycling occurring below the ground, climate change causes an increase in plant biomass, height, cover, and abundance, a process often referred to as Arctic greening (Myers-Smith et al., 2020). Arctic greening has been studied

as spectral greening through satellite-derived vegetation indices and as vegetation greening through field observed changes in vegetation. It is a heterogeneous process that is affected by a variety of ecological and environmental factors. Ecological factors that promote greening include (but are not limited to) changing plant community composition (e.g. Forbes et al., 2010), changing plant traits (e.g. Bjorkman et al., 2018), and colonization of previously non-vegetated areas (Elmendorf et al., 2012). Environmental factors that affect greening include (but are not limited to) topography (Riihimäki et al., 2017), changing snowmelt dynamics and soil moisture (Gamon et al., 2013; Semenchuk et al., 2016) and soil nutrient levels (Gu & Grogan, 2020). Some regions of the Arctic have experienced browning rather than greening, though browning occurs to a much lesser extent. Studies have shown the extent of landscape greening to vary between 13-42% and landscape browning from 1-4% (Myers-Smith et al., 2020; Park et al., 2016). Arctic browning is also caused by both environmental and ecological factors, such as large-scale defoliation due to extreme climatic events (e.g. Bjerke et al., 2017), altered landscape hydrology (Raynolds & Walker, 2016; Smith et al., 2005) and outbreaks of herbivory or pathogens (Lund et al., 2017).

Although the Arctic is generally greening, the changes in the community structure, traits, phenology, and ecology of Arctic plants are heterogeneous and complex. The most common result in warming experiments and long-term observational studies of plant community composition is that of no change, indicating a resilience to the current extent of Arctic climate change. However, where change is occurring, graminoids and shrubs are likely to respond positively to warming, whereas lichens and bryophytes usually respond negatively (Bjorkman et al., 2020).

Graminoids, being a potential beneficiary of climate change, and often being associated with AM, also a potential beneficiary of climate change, make the graminoid–AM association particularly important as it could affect the way in which the Arctic plant community composition changes in response to climate change. It has been shown that AM–associated species have both higher growth rates and produce litter that decomposes more rapidly than EM and ERM–associated species (J. H. C. Cornelissen et al., 2001). Additionally, AM associated plants allocate a smaller proportion of their carbon to their fungal symbionts than EM plants, potentially affecting the amount of carbon stored below ground long term (Soudzilovskaia et al., 2015). A shift in the plant community, and their associated fungi, could therefore have large effects on the carbon balance of the Arctic tundra. Therefore, understanding the effect of increasing temperatures on the prevalence of the graminoid–AM association would give additional insight into the impact of climate change on the carbon balance in the Arctic.

Aim

This thesis aims to explore if 1; AM colonization in the Arctic graminoid *Poa alpina* increases with increasing temperatures, 2; AM colonization in *P. alpina* increases with decreasing P:N ratio, 3; shoot nutrient content in *P. alpina* increases with increasing AM colonization, and 4; the positive response of AM associated graminoids to increasing temperatures could be related to their AM association.

Method

Field sampling

In August 2021 the AM associated graminoid *Poa alpina* (Read & Haselwandter, 1981) was collected from 12 plots at the Latnjajaure research station in northern Sweden. The plots were distributed across an altitudinal gradient from ~950 to 1250 m a.s.l. and were chosen based on

the presence of *P. alpina*. Due to the ecological preferences of *P. alpina*, often growing below melting snowbeds or along streams, the plots were mesic or wet. All plots had a TMS-4 datalogger recording temperature data (Wild et al., 2019) (hereafter "TMS-logger") located at the center of the plot and all *P. alpina* samples were collected within a 20 meters radius from the center. The TMS-loggers collected temperature data 8 cm below ground (BGT), at the ground surface (GST) and 15 cm above the surface (AGT) at ten-minute intervals. The loggers were placed in the field in the beginning of June 2020 and temperature data were therefore available for 14 months. 15 specimens of *P. alpina* were collected from each plot. The entire specimen (shoot and roots) was collected, and the roots were cleaned of soil and debris by hand immediately. The samples were then stored in 45% isopropanol at 15 ˚C for ~7 days and below 4˚C long term.

Soil samples of 200 ml were also collected from all plots consisting of 5-10 subsamples, (amount depending on soil depth), and were collected with a soil auger (3 cm Ø). The soil samples were homogenized and stored below 4˚C for ~7 days and then kept frozen at ca. -20 ˚C. The soil subsamples were also collected within the 20 m radius from the plot center.

Nutrient analysis

The shoots (all growth above the highest root) were dried at 60 ˚C for 48 h and ball milled. These pooled for the entire plot and analyzed for total N and P externally (IGN/Geography IRMS lab, University of Copenhagen, Denmark). Additionally, 10g (dry weight) subsamples of each soil sample were also dried and sieved (0.6 mm) and analyzed for inorganic P externally (IGN/Geography IRMS lab, University of Copenhagen, Denmark). The remaining soil samples were kept frozen and were sent for analysis of inorganic N at the soil and plant laboratory, SLU, Sweden.

Mycorrhizal root colonization

To determine the extent of AM colonization of *P. alpina* the roots were cut in to 1 cm segments and stained with pelican blue ink according to the protocol by Vierheilig et al. (1998) due to its benefits over alternative methods regarding staining results, cost and importantly the toxicity of compounds used (Vierheilig et al., 2005). This procedure clears the plant roots and colors the fungal structures (hyphae, arbuscules and vesicles) blue (results visible in fig. 1, pale blue root and thin dark blue AM hyphae). Root AM colonization was determined by mounting 15-20 well stained root segments on a petri dish and visually estimating their colonization density according to six categories (figure 2) as described by Biermann and Linderman (1981) implementing the modifications by Trouvelot et al. (1986). This visual estimation of AM colonization density only took hyphae into account (and not arbuscules and vesicles), as the staining success did not allow for reliable determination of arbuscules and vesicles. The categories were defined as no AM structures (0), < 1% coverage of AM structures (1), < 10% coverage of AM structures and (2), < 50% coverage of AM structures (3), < 80% coverage of AM structures (4) and > 80% coverage of AM structures (5). Mycorrhizal density was scored under a stereo microscope with 20-40x magnification (figure 1).

Data analyses

The data from the TMS-loggers was used to calculate the sum of temperatures above 3 ˚C (T_{sum}) for each plot from june 2020 to august 2021. This is a measure of the cummulative buildup of heat above 3 ˚C and takes both the length and intensity of the growth season into

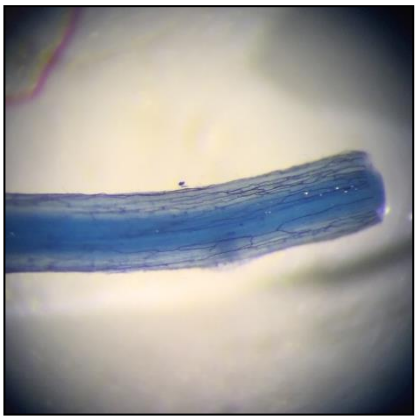

Figure 1. A stained root segment of *Poa alpina* colonized by arbuscular mycorrhizal hyphae. Hyphae are thin dark blue strands on pale blue root.

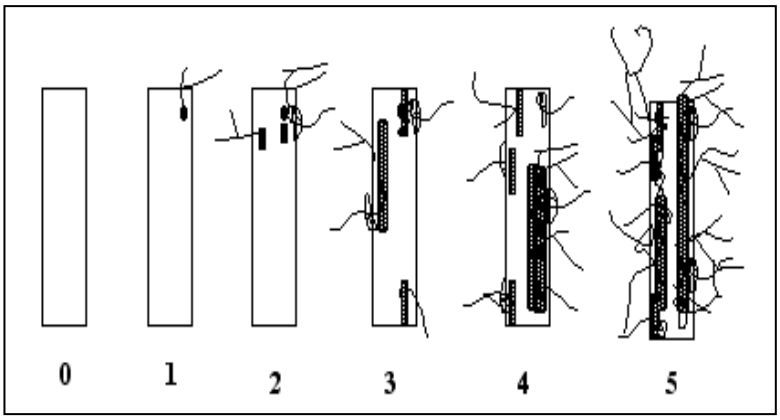

Figure 2. The classification system used to estimate mycorrhizal colonization density on stained segments of *Poa alpina* roots as described by Biermann and Linderman (1981) implementing the modifications by Trouvelot et al. (1986).

account. Furthermore T_{sum} was normalized for each plot and measurement depth following these formulas:

$$\mathrm{BGT} = (T_{\mathrm{sum-8cm}} - mean_{\mathrm{Tsum-8cm}}) \div SD_{\mathrm{Tsum-8cm}}$$
$$\mathrm{GST} = (T_{\mathrm{sum0cm}} - mean_{\mathrm{Tsum0cm}}) \div SD_{\mathrm{Tsum0cm}}$$
$$\mathrm{AGT} = (T_{\mathrm{sum15cm}} - mean_{\mathrm{Tsum15cm}}) \div SD_{\mathrm{Tsum15cm}}$$

This was done as the values of T_{sum} were very large and difficult for the statistical program to handle. The effect of BGT, GST and AGT on arbuscular myccorhizal colonization was tested with a cummulative ordinal regression model (ORM) (Bürkner & Vuorre, 2019) in R (R Core Team, 2021) with the tidyverse (Hadley Wickham, 2019), brms (Bürkner, 2021) and ggplot2 (Wickham, 2016) packages. This model tests how changes in BST, SST and AST effect the probablitiy of observed colonization classes. It does so by assuming that the observed ordinal variable (the AM colonization class), originates from the categorization of a non observable continuous variable (estimated AM colonization). This latent variable is assumed to be normally distributed and have a standard deviation (SD) of one.

The relationships between mycorrhizal colonization, temperature and soil N, soil P, tissue N and tissue P were tested with linear and multiple regressions in R (R Core Team, 2021), with the ggplot2 (Wickham, 2016), dplyr (Wickham et al., 2022), broom (Robinson et al., 2022) and ggpubr (Kassambara, 2020), cowplot (Wilke, 2020) and scales (Wickham & Seidel, 2022) packages. Sharpio Wilks tests, histograms and residual plots were used to determine normal distribution and homogeneity of variance. As the multiple regression testing the effect of AM colonization, temperature and the corresponding soil nutrient required a single value per plot for each independent variable, the colonization classes of the root segments were insufficient, and the effect of mycorrhizal colonization was tested on the plot average of the estimated AM colonization generated by the OLR. The full scripts can be seen in Appendix C.

Results

Root AM colonization

From all specimens analysed 297 (9,7%) root segments were classified as class 0, 640 (20,9%) were classified as 1, 803 (26,2%) were classified as 2, 1137 (37,1%) were classified as 3, 167 (5,4%) were classified as 4 and 23 (0,7%) were classified as class 5 (Figure 3).

The ORM tests how the probability of a class changes with changes in temperature. The slope of BGT was 0,23 with a 95% confidence intervall (CI) from -0,44 to 0,87 (Figure 4). This indicated that an increase in one SD of BGT was likley to increase the average of the latent continous variable (estimated AM colonization) by 0,23 SD (i.e. have a positive effect on mycorhizal colonization). However, due to the wide confidecne interval it could also have a negative or neutral effect on mycorrhizal colonization. Additionally, a hypothesis test showed the probability of a positive effect of BGT on mycorrhizal colonization to be 75% (Table 1). Figure 4A shows how the probabilities of each class change in response to BGT. GST had a regression coefficiant of 0,54, with a 95% CI from -0,04 to 1,15. The effect of GST on mycorhizal colonization was therefore likley to be positive, or neutral. This was also supported by a probability of a positive effect of 97% (Table 1). The effect of GST on the probabileties of each class are shown in figure 4B. AST had a slope of 0,42 with a 95% CI from -0,18 to 1,03. The effect of AST is therfore likley to be positive, but could range from negative to positive. The probability of a positive effect of AGT was 92% (Table 1). The effect of GST on the probabileties of each class are shown in figure 4C. Aditionally, all temperature measurements were tested for interactive effects with the soil nutrients, but no interactions were detected (Appendix B, Table 1). Soil P had a slope of -0,30 with a 95 CI from -0,93 to 0,31 and a 84% probability of having a negative effect on root AM colonization. Soil N (ammonium, the levels of nitrate were also anlayzed but were to low for reliable quantification) had a slope of -0,19 with a 95 CI from -0,90 to 0,49 and a 31% probability of having a positive effect on root AM colonization (Table 1). Interactive effects between temperature and soil nutrients were not found (Appendix B, Table 1).

Table 1. Statistical analyses of the relationship between arbuscular mycorrhizal colonization and below ground temperature, soil surface temperature, above ground temperature soil P and soil N. The lower and upper confidence intervals around the slope show the certainty of the ordinal regression model. The hypothesis test shows the probability of a positive effect on arbuscular mycorrhizal colonization of below ground temperature, ground surface temperature, above gound temperature and soil N, and an negative effect of soil P on mycorrhizal colonization of *Poa alpina* roots.

Variable	Lower 95% confidence intervall	Slope	Upper 95% confidence intervall	Hypothesis test
Below ground temperature	-0,44	0,23	0,87	75%
Ground surface temperatrue	-0,04	0,54	1,15	97%
Above gound temperature	-0,15	0,44	1,06	92%
Soil P	-0.98	-0.31	0.39	84%
Soil N	-0,88	-0.17	0.54	31%

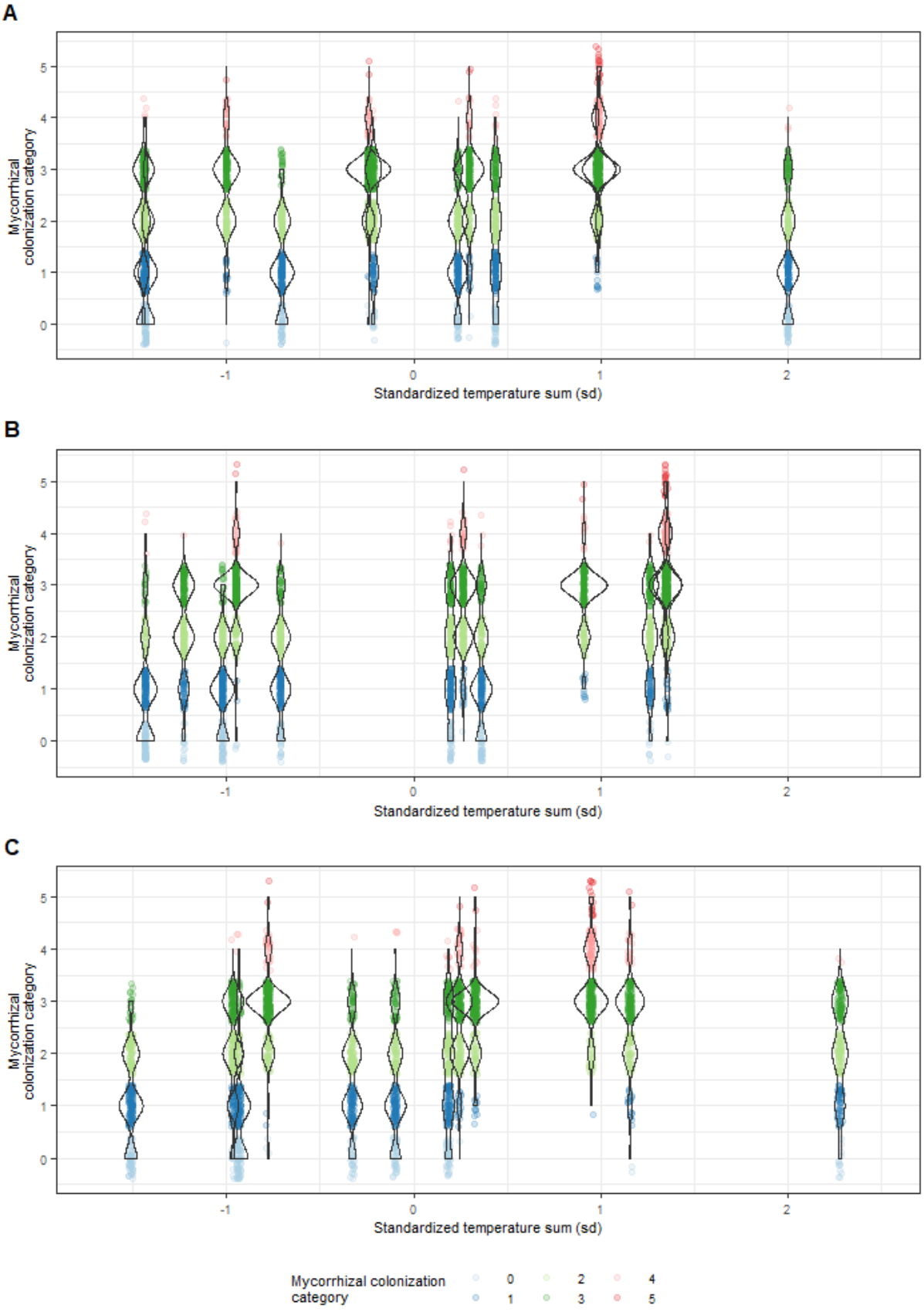

Figure 3. Shows the spread of root colonization for all 12 plots and orders the plots according to **A** below ground temperature, **B** ground surface temperature and **C** above ground temperature. The temperature is standardized and varies in standard deviations around the mean. Points show the colonization of 1 cm root segments, and violin with shows density of points.

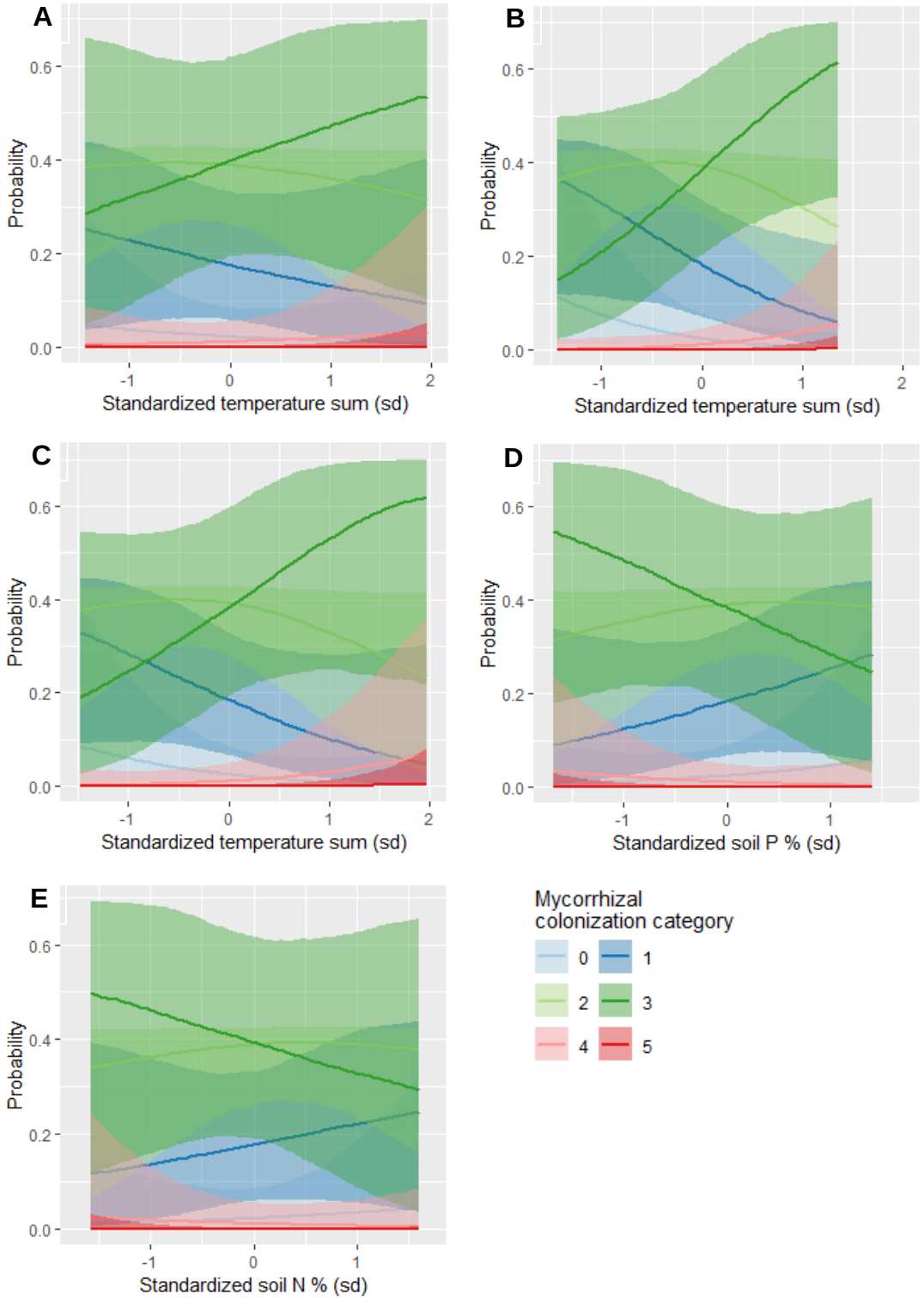

Figure 4. An ordinal logistic regression that shows how the probability of a certain colonization category changes with changing below ground temperatures. **A** shows the effect of below ground temperature on mycorrhizal colonization, the slope was 0,23 with a 95% confidence interval (CI) from -0,44 to 0,87. **B** shows the effect of ground surface temperature on mycorrhizal colonization, the slope was 0,0,54 with a 95% CI from -0,04 to 1,15. **C** shows the effect of above ground temperature on mycorrhizal colonization, the slope was 0,44 with a 95% CI from -0,15 to 1,06. The temperature is standardized and varies in standard deviations around the mean. **D** shows the effect of soil P on mycorrhizal colonization, the slope was -0,31 with a 95% CI from -0,98 to 0,39. The temperature is standardized and varies in standard deviations around the mean. **E** shows the effect of above ground temperature on mycorrhizal colonization, the slope was -0,17 with a 95% CI from -0,88 to 0,54. The temperature is standardized and varies in standard deviations around the mean.

Tissue nutrients

Tissue N (all nutrients presented in percent dry mass) showed a significant and slightly negative response to estimated AM colonization in the three tests performed (Table 2). However, neither of the temperature measurements or soil N had a significant effect on tissue N. All tests were able to explain more than half of the variance in tissue N (R^2 = 0,53-0,59, f-test = 0,02-0,03). A strong negative relationship was found between tissue N and AM colonization (R^2 = -0,16, p = 0,01) (Figure 5). Tissue P did not show any significant relationships with any of the temperature measurements, soil P or estimated AM colonization (Table 2, Figure 6). Although insignificant, a potential positive trend between soil P and tissue P was seen (R^2 = 0,79, p = 0,06, f-test = 0,23-0,24). Interactions between the independent variables were not included in the regressions as they did not improve the fit of the model.

Table 2. The results from six multiple regression analyses that tested the relationship between tissue N% and tissue P% (both presented in percent dry mass) and below ground temperature (BGT), ground surface temperature (GST), above ground temperature (AGT), their corresponding soil nutrient and the estimated arbuscular mycorrhizal (AM) colonization. No significant relationships were found between tissue N and any of the temperature measurements or soil N while a negative, significant relationship was found between tissue N and estimated AM colonization. Additionally, more than half of the observed variance in tissue N could be explained by all three multiple linear regressions and the significant f-test confirms this. No significant relationships were found between tissue P and BGT, GST, AGT, soil P or estimated AM colonization, although a positive trend between increasing soil P and increasing tissue P was observed (R^2 = 0,79, p = 0,06), this was insignificant (f-test = 0,23-0,24).

Relationship	Slope	p-value	Adjusted R^2
Tissue N%			
x BGT	-0,05	0,27	
x Soil N%	-2,32	0,95	
x Estimated AM colonization	-0,16	0,01*	
F-test		0,03*	0,54
Tissue N%			
x GST	-0,06	0,27	
x Soil N%	7,90	0,82	
x Estimated AM colonization	-0,13	0,05*	
F-test		0,03*	0,54
Tissue N%			
x AGT	-0,08	0,14	
x Soil N%	17,14	0,61	
x Estimated AM colonization	-0,13	0,03*	
F-test		0,02*	0,59

Tissue P%			
x BGT	0,00	0,99	
x Soil P%	0,79	0,06	
x Estimated AM colonization	0,00	0,32	
F-test		0,24	0,17
Tissue P%			
x GST	-0,00	0,91	
x Soil P%	0,77	0,07	
x Estimated AM colonization	0,00	0,34	
F-test		0,24	0,17
Tissue P%			
x AGT	-0,00	0,80	
x Soil P%	0,74	0,09	
x Estimated AM colonization	0,00	0,29	
F-test		0,23	0,17

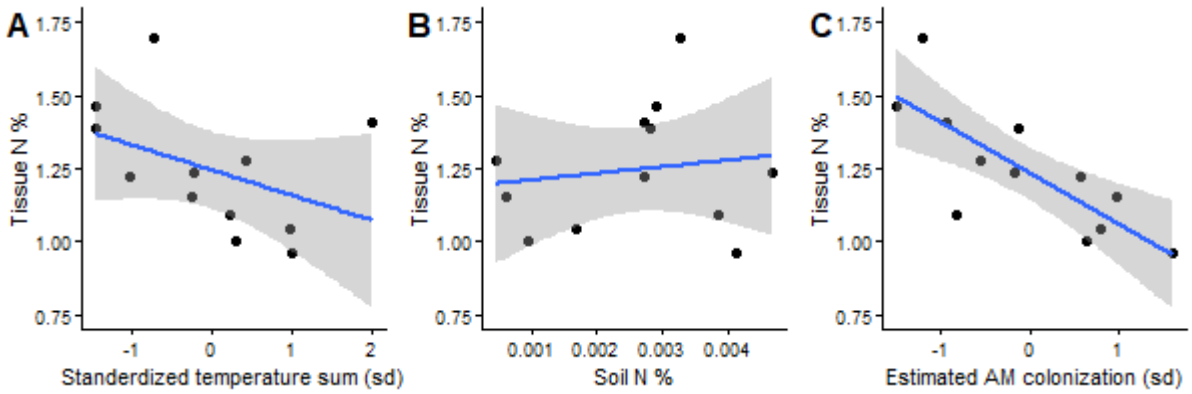

Figure 5. Multiple regression analysis of relationship between plot temperature (standardized and varying in standard deviations around the mean), soil N% (presented in percent dry mass) and the estimated arbuscular mycorrhizal (AM) colonziation and tissue N%. The multiple regression explained more than half of the variance of tissue N% (R^2 = 0,54) **A** Shows the relationship between tissue N% and below ground temperature. No significant trend was found (p = 0,27, slope = -0,05). **B** The relationship between tissue N% and soil N%. No significant relationship was found (p = 0,95, slope = -2,32). **C** The relationship between tissue N% and estimated AM colonization. The regression was significant (p = 0,01) and negative (slope = -0,16).

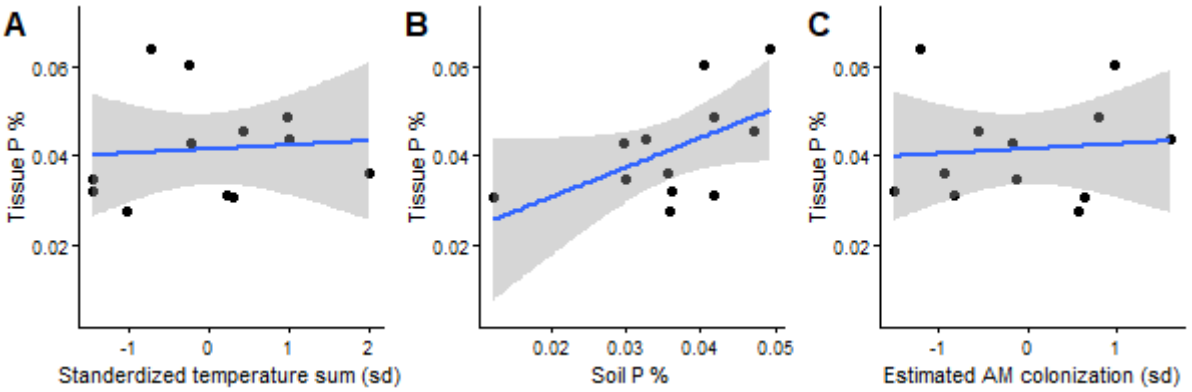

Figure 6. Multiple regression analysis of relationship between plot temperature (standardized and varying in standard deviations around the mean), soil P% (presented in percent dry mass) and the estimated arbuscular mycorrhizal (AM) colonziation and tissue P%. The multiple regression explained 17% of the variance of tissue P%. **A** Shows the relationship between tissue P% and below ground temperature. No significant trend was found (p = 0,99, slope = 0,00). **B** The relationship between tissue P% and soil P%. A potentially positive trend was found (p = 0,06, slope = 0,79). **C** The relationship between tissue P% and estimated AM colonization. No significant trend was observed (p = 0,32, slope = 0,00).

Discussion

Root AM colonization

There was a likely overall positive relationship between warmer temperatures and increased AM colonization of *P. alpina* (Figure 4A, 4B and 4C), but the large confidence intervals show that the degree of this positive relationship is very uncertain. Depending on where the temperature is measured, 8 cm below ground, at the ground surface or 15 cm above the ground, there was respectively a 75%, 97% and 92% probability of a positive relationship between increasing temperatures and a higher degree of AM colonization (Table 1). The infrequency of AM colonization at low temperatures indicates that the temperature limitations of AM are a likely contributor to their relatively low prevalence in the Arctic. These results are also in line with the positive relationship previously found between temperature and the AM association with various Arctic forb species, such as *Gnaphalium norvegicum*, *Artemisia tridentata* and *Ranunculus acris* (Kytoviita & Ruotsalainen, 2007; Rudgers et al., 2014; A. L. Ruotsalainen & Kytöviita, 2004), and indicate that a similar relationship between graminoids and AM is likely. However, as the relationship between AM and graminoids has seldom been the focus of research, these results are quite novel. Rudgers et al. (2014) found the AM colonization of high altitude graminoids *Achnatherum lettermanii* to increase in response to experimental warming, while *Poa pratensis* and *Festuca thurberi* saw no change in overall colonization but large increases in arbuscule abundance, showing the importance of alternative AM quantification methods that have the ability to reliably quantify al AM structures (hyphae, arbuscules and vesicles), and not only fungal hyphae. Due to the magnification used and successes of the staining procedure this analysis was only able to quantify hyphal density. However, De Long et al. (2015) found a negative relationship between temperature and AM colonization in the low Arctic graminoids *Deschampsia flexuosa* and *Festuca ovina*. The authors theorized that the value of AM is highest for the most nutrient limited plants (i.e., plants found in colder temperatures). However, these contradictory results and hypotheses highlights the necessity to further study the relationship between the graminoid–AM association, temperature, and soil conditions.

Because the mycorrhizal fungi are active below ground their activity was expected be closely linked to soil temperatures (BGT) and less closely to soil surface (GST) or above ground (AGT) temperatures. That the positive relationship between AM colonization and temperature was more

uncertain for BGT than for the other temperature types can in part explained by a single plot which had high soil temperatures but low levels of AM colonization (Figure 3A). A stronger relationship between BGT and AM colonization would be expected if this study were executed at a greater scale (i.e., larger sample sizes and/or larger altitudinal gradient). In addition, the positive correlations between AM and temperature would likely have been stronger with the use of a larger altitudinal gradient, which is supported by Anna Liisa Ruotsalainen et al. (2002) who noted that studies using altitudinal gradients > 1000m have been more likely to find relationships between mycorrhizal association and temperature.

In contrast to my hypothesis, there was no significant relationship between AM colonization and changes in P or N. This could be due to the small altitudinal gradient and limited sample size of this project, as a positive trend was seen between decreasing P availability and increasing AM colonization (Table 1, Figure 4D). However, no relationship between increasing N availability and AM colonization was observed (Table 1). That decreasing P availability could affect AM colonization more than N availability corresponds well with the knowledge about the plant–AM association. Although AM are active in both N and P uptake, their importance as a N provider is uncertain (Hodge & Storer, 2015) and their main function is typically as a provider of plant P. Therefore, a climate change driven change in the soil conditions, in part with increasing pH and a related decrease in P mobility is expected to increase the benefit of AM association (Read, 1991). However, AM of arctic and boreal biomes are understudied, and climactic zones and ecosystems have been shown to impact their community composition (Öpik et al., 2010; Öpik et al., 2013), and assuming functional redundancy between fungal communities in different environments might overlook potential adaptations of Arctic AM to local conditions. Therefore, further research into AM community composition, and their ecological functions is necessary to increase our understanding of the Arctic plant–AM association, and to what extent Arctic AM have adapted to their environmental conditions.

Additionally, for continued research to quantify how AM biomass, and the biomass of AM associated graminoids will respond to climate change should be prioritized as it would bridge the knowledge gap between Arctic graminoid–AM associations and their potential impact on Arctic carbon cycling. It is known that AM associated plants generally allocate less carbon below ground than ERM and EM associated plants, and AM fungi typically contain less melanin than ERM and EM fungi, making them less resistant to decomposition (Soudzilovskaia et al., 2015). Additionally, AM associated plants are generally adapted to high nutrient soils and tend to have fast leaf economics (e.g., through high leaf nutrient concentrations). An effect of their fast leaf economics is that they decompose rapidly compared to ERM and EM associated plants (J. H. C. Cornelissen et al., 2001). Contrarily, ERM and EM fungi tend to decrease soil organic matter decomposition by scavenging for nitrogen in soil organic matter, increasing the soil C:N ratio (Soudzilovskaia et al., 2015). The characteristics of the AM, ERM and EM fungal associations indicate that an ecosystem where AM associated plants (such as graminoids) become more prevalent, potentially at the expense of ERM and EM associated plants, could lose soil carbon. Due to the large amount of carbon stored in Arctic soils, a shift in the Arctic carbon balance could have global implications.

Tissue nutrients

Due to the uncertainty regarding the N uptake of AM (Hodge & Storer, 2015) one of two trends in tissue nitrogen was expected. If AM were not important for the N uptake of *P. alpina* a positive relationship between tissue N and soil N was expected as the plants absorb their nitrogen directly from the soil. However, if AM were important for the N uptake of *P. alpina* a positive relationship between tissue N and AM colonization was expected. Instead, the results showed a negative relationship between AM colonization and tissue N, and no significant relationship between tissue N and soil N (Table 2, Figure 5). The negative relationship between tissue N and AM colonization could be caused by the temperature limitation of AM making the association a poor investment of

carbon for plants with sufficient N availability. As the temperature limitations of AM likely limit their carbon use efficiency (i.e., they require relatively large amounts of carbon to supply their host plants with N), plants with sufficient N availability invest their carbon in other traits to increase their fitness. For plants with low N availability however, AM association might be a beneficial investment regardless of its inefficiency. Although the limited scale of this study (n=12) limits the certainty of these conclusions they correspond well to the results previously presented by De Long et al. (2015).

No significant relationships were found between tissue P and temperature, soil P or AM colonization, although a positive relationship between tissue P and soil P was observed (Table 2, Figure 6). The lack of a relationship between tissue P and AM colonization was unexpected, as AM are foremost associated with the supply of P to their plant hosts. However, if P was not the main growth-limiting nutrient for the specimens of *P. alpina*, the nutrient being relatively mobile in the low soil pH, and instead nitrogen was the main nutrient limiting growth (Read, 1991; Read & Perez-Moreno, 2003), the stronger relationship between tissue N and AM colonization potentially indicates that the Arctic plant–AM association might be largely centered around the exchange of carbon and N, and not carbon and P. However, the limited scale of this study, and that this theory does not correspond with the previously discussed results showing a negative relationship between soil P and AM colonization and no relationship between soil N and AM colonization indicate that Arctic AM are active in the supply of P to their plant hosts. Due to the results indicating AM activity in both P and N uptake, it is important for continued research to quantify the benefit of AM for tissue P and tissue N to fully discern the value of AM association is for the nutrient uptake of Arctic graminoids in different soil conditions.

Conclusions

This thesis found a likely increase in the AM colonization of the Arctic graminoid *Poa alpina* with increasing temperature, adding to the scarce knowledge regarding how the Arctic graminoid–AM is affected by changes in temperature. Additionally, a decreasing content of mineralized soil phosphorus was potentially related to an increase in AM colonization. However, no relationship between AM colonization and tissue P was observed, while a strong negative relationship was found between AM colonization and tissue N, possibly indicating that AM association is most valuable for the most N limited plants. Although discerning to what extent the P and N supply of *P. alpina* benefits from AM association was not clear in this study, the increased colonization in response to increasing temperatures indicate that as climate change continues to warm the Arctic, the fungal associates of some Arctic graminoids become more beneficial to their hosts. Therefore, their AM association is likely to play a role in the fitness and competitive relationship between these graminoids and other Arctic plant life and the harsh arctic environment.

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References

Allen, E. B., Chambers, J. C., Connor, K. F., Allen, M. F., & Brown, R. W. (1987). Natural reestablishment of mycorrhizae in disturbed alpine ecosystems. *Arctic and alpine research, 19*(1), 11-20. doi:10.2307/1550995

Berg, B. (2018). Decomposing litter limit values humus accumulation, locally and regionally. *Applied soil ecology : a section of Agriculture, ecosystems & environment, 123*, 494-508. doi:10.1016/j.apsoil.2017.06.026

Biermann, B., & Linderman, R. G. (1981). Quantifying Vesicular-Arbuscular Mycorrhizae: A Proposed Method Towards Standardization. *The New phytologist, 87*(1), 63-67. doi:10.1111/j.1469-8137.1981.tb01690.x

Bjerke, J. W., Treharne, R., Vikhamar-Schuler, D., Karlsen, S. R., Ravolainen, V., Bokhorst, S., . . . Tømmervik, H. (2017). Understanding the drivers of extensive plant damage in boreal and Arctic ecosystems: Insights from field surveys in the aftermath of damage. *The Science of the total environment, 599-600*(-), 1965-1976. doi:10.1016/j.scitotenv.2017.05.050

Bjorkman, A. D., Garcia Criado, M., Myers-Smith, I. H., Ravolainen, V., Jonsdottir, I. S., Westergaard, K. B., & Lawler, J. P. (2020). Status and trends in Arctic vegetation: Evidence from experimental warming and long-term monitoring.(Report). *Ambio, 49*(3), 678. doi:10.1007/s13280-019-01161-6

Bjorkman, A. D., Myers-Smith, I. H., Elmendorf, S. C., Ozinga, W. A., Sheremetev, B., Weiher, E., & et al. (2018). Plant functional trait change across a warming tundra biome. *Nature (London), 562*(7725), 57-62. doi:10.1038/s41586-018-0563-7

Bledsoe, C., Klein, P., & Bliss, L. C. (1990). A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. *Canadian journal of botany, 68*(9), 1848-1856. doi:10.1139/b90-242

Bürkner, P.-C. (2021). Bayesian Item Response Modeling in R with brms and Stan. *Journal of Statistical Software, 100*(5), 1-54. doi:10.18637/jss.v100.i05

Bürkner, P.-C., & Vuorre, M. (2019). Ordinal Regression Models in Psychology: A Tutorial. *Advances in methods and practices in psychological science, 2*(1), 77-101. doi:10.1177/2515245918823199

Chapin, F. S., & Shaver, G. R. (1985). Individualistic Growth Response of Tundra Plant Species to Environmental Manipulations in the Field. *Ecology (Durham), 66*(2), 564-576. doi:10.2307/1940405

Christiansen, C. T., Haugwitz, M. S., Priemé, A., Nielsen, C. S., Elberling, B., Michelsen, A., . . . Blok, D. (2017). Enhanced summer warming reduces fungal decomposer diversity and litter mass loss more strongly in dry than in wet tundra. *Global Change Biology, 23*(1), 406-420. doi:10.1111/gcb.13362

Cornelissen, J. H. C., Aerts, R., Cerabolini, B., Werger, M. J. A., & van der Heijden, M. G. A. (2001). Carbon Cycling Traits of Plant Species Are Linked with Mycorrhizal Strategy. *Oecologia, 129*(4), 611-619. doi:10.1007/s004420100752

Cornelissen, J. H. C., Van Bodegom, P. M., Aerts, R., Callaghan, T. V., Van Logtestijn, R. S. P., Alatalo, J., . . . Team, M. O. L. (2007). Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. *Ecology Letters, 10*(7), 619-627. doi:https://doi.org/10.1111/j.1461-0248.2007.01051.x

Dalpé, Y., & Aiken, S. G. (1998). Arbuscular mycorrhizal fungi associated with Festuca species in the Canadian High Arctic. *Canadian journal of botany, 76*(11), 1930-1938. doi:10.1139/b98-165

De Long, J. R., Kardol, P., Sundqvist, M. K., Veen, G. F., & Wardle, D. A. (2015). Plant growth response to direct and indirect temperature effects varies by vegetation type and elevation in a subarctic tundra. *Oikos, 124*(6), 772-783. doi:10.1111/oik.01764

Elmendorf, S. C., Henry, G. H. R., Hollister, R. D., Bjork, R. G., Boulanger-Lapointe, N., Cooper, E. J., . . . Wipf, S. (2012). Plot-scale evidence of tundra vegetation change and links to recent summer warming. *Nature Climate Change, 2*(6), 453-457. doi:10.1038/nclimate1465

Forbes, B. C., Fauria, M. M., & Zetterberg, P. (2010). Russian Arctic warming and 'greening' are closely tracked by tundra shrub willows. *Global Change Biology, 16*(5), 1542-1554. doi:10.1111/j.1365-2486.2009.02047.x

Gamon, J. A., Huemmrich, K. F., Stone, R. S., & Tweedie, C. E. (2013). Spatial and temporal variation in primary productivity (NDVI) of coastal Alaskan tundra: Decreased vegetation growth following earlier snowmelt. *Remote Sensing of Environment, 129*, 144-153. doi:https://doi.org/10.1016/j.rse.2012.10.030

Giesemann, P., Eichenberg, D., Stöckel, M., Seifert, L. F., Gomes, S. I. F., Merckx, V. S. F. T., . . . Bennett, A. (2020). Dark septate endophytes and arbuscular mycorrhizal fungi (Paris-morphotype) affect the stable isotope composition of 'classically' non-mycorrhizal plants. *Functional ecology, 34*(12), 2453-2466. doi:10.1111/1365-2435.13673

Gu, Q., & Grogan, P. (2020). Responses of low Arctic tundra plant species to experimental manipulations: Differences between abiotic and biotic factors and between short- and long-term effects. *Arctic, Antarctic, and Alpine Research, 52*(1), 524-540. doi:10.1080/15230430.2020.1815360

Hadley Wickham, M. A., Jennifer Bryan, Winston Chang, Lucy D'Agostino McGowan, Romain François, Garrett Grolemund, Alex Hayes, Lionel Henry, Jim Hester, Max Kuhn, Thomas Lin Pedersen, Evan Miller, Stephan Milton Bache, Kirill Müller, Jeroen Ooms, David Robinson, Dana Paige Seidel, Vitalie Spinu, Kohske Takahashi, Davis Vaughan, Claus Wilke, Kara Woo and Hiroaki Yutani. (2019). Welcome to the tidyverse. *Journal of Open Source Software, 4*(43), 1686. doi:10.21105/joss.01686

Haselwandter, K., & Read, D. J. (1982). The Significance of a Root-Fungus Association in Two Carex Species of High-Alpine Plant Communities. *Oecologia, 53*(3), 352-354. doi:10.1007/BF00389012

Hill, P. W., Broughton, R., Bougoure, J., Havelange, W., Newsham, K. K., Grant, H., . . . Knops, J. (2019). Angiosperm symbioses with non-mycorrhizal fungal partners enhance N acquisition from ancient organic matter in a warming maritime Antarctic. *Ecology Letters, 22*(12), 2111-2119. doi:10.1111/ele.13399

Hobbie, S. E. (1996). Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecological monographs, 66*(4), 503-522. doi:10.2307/2963492

Hobbie, S. E., Eddy, W. C., Buyarski, C. R., Adair, E. C., Ogdahl, M. L., & Weisenhorn, P. (2012). Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. *Ecological monographs, 82*(3), 389-405. doi:10.1890/11-1600.1

Hodge, A., & Storer, K. (2015). Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. *Plant and soil, 386*(1/2), 1-19. doi:10.1007/s11104-014-2162-1

IPCC. (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Retrieved from https://www.ipcc.ch/report/ar6/wg1/#FullReport

Kassambara, A. (2020). ggpubr: 'ggplot2' Based Publication Ready Plots. Retrieved from https://CRAN.R-project.org/package=ggpubr

Kauppinen, M., Raveala, K., Wäli, P. R., & Ruotsalainen, A. L. (2013). Contrasting preferences of arbuscular mycorrhizal and dark septate fungi colonizing boreal and subarctic Avenella flexuosa. *Mycorrhiza, 24*(3), 171-177. doi:10.1007/s00572-013-0526-7

Kohn, L. M., & Stasovski, E. (1990). The mycorrhizal status of plants at Alexandra Fiord, Ellesmere Island, Canada, a High Arctic site. *Mycologia, 82*(1), 23-35. doi:10.2307/3759959

Kytoviita, M.-M. (2005). Asymmetric symbiont adaptation to Arctic conditions could explain why High Arctic plants are non-mycorrhizal. *FEMS microbiology ecology, 53*(1), 27-32. doi:10.1016/j.femsec.2004.09.014

Kytoviita, M.-M., & Ruotsalainen, A. L. (2007). Mycorrhizal benefit in two low arctic herbs increases with increasing temperature. *American journal of botany, 94*(8), 1309-1315. doi:10.3732/ajb.94.8.1309

Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., . . . Li, B. (2013). Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. *Ecology, 94*(3), 726-738. doi:https://doi.org/10.1890/12-0279.1

Lund, M., Raundrup, K., Westergaard-nielsen, A., López-blanco, E., Nymand, J., & Aastrup, P. (2017). Larval outbreaks in West Greenland: Instant and subsequent effects on tundra ecosystem productivity and CO2 exchange. *Ambio, 46*, 26-38. doi:http://dx.doi.org/10.1007/s13280-016-0863-9

Myers-Smith, I. H., Kerby, J. T., Phoenix, G. K., Bjerke, J. W., Epstein, H. E., Assmann, J. J., . . . Forbes, B. C. (2020). Complexity revealed in the greening of the Arctic. *Nature Climate Change, 10*(2), 106-117. doi:10.1038/s41558-019-0688-1

Newsham, K. K. (2011). A meta-analysis of plant responses to dark septate root endophytes. *The New phytologist, 190*(3), 783-793. doi:10.1111/j.1469-8137.2010.03611.x

Newsham, K. K., Upson, R., & Read, D. J. (2009). Mycorrhizas and dark septate root endophytes in polar regions. *Fungal ecology, 2*(1), 10-20. doi:10.1016/j.funeco.2008.10.005

Park, T., Ganguly, S., Tømmervik, H., Euskirchen, E. S., Høgda, K.-A., Karlsen, S. R., . . . Myneni, R. B. (2016). Changes in growing season duration and productivity of northern vegetation inferred from long-term remote sensing data. *Environmental research letters, 11*(8), 84001. doi:10.1088/1748-9326/11/8/084001

Püschel, D., Bitterlich, M., Rydlová, J., & Jansa, J. (2020). Facilitation of plant water uptake by an arbuscular mycorrhizal fungus: a Gordian knot of roots and hyphae. *Mycorrhiza, 30*(2-3), 299-313. doi:10.1007/s00572-020-00949-9

R Core Team. (2021). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/.

Raynolds, M. K., & Walker, D. A. (2016). Increased wetness confounds Landsat-derived NDVI trends in the central Alaska North Slope region, 1985-2011. *Environmental research letters, 11*(8), 85004. doi:10.1088/1748-9326/11/8/085004

Read, D. J. (1991). Mycorrhizas in ecosystems. *Experientia, 47*(4), 376-391. doi:10.1007/BF01972080

Read, D. J., & Haselwandter, K. (1981). Observations on the Mycorrhizal Status of Some Alpine Plant Communities. *The New phytologist, 88*(2), 341-352. doi:10.1111/j.1469-8137.1981.tb01729.x

Read, D. J., & Perez-Moreno, J. (2003). Mycorrhizas and Nutrient Cycling in Ecosystems: A Journey towards Relevance? *The New phytologist, 157*(3), 475-492. doi:10.1046/j.1469-8137.2003.00704.x

Riihimäki, H., Heiskanen, J., & Luoto, M. (2017). The effect of topography on arctic-alpine aboveground biomass and NDVI patterns. *International Journal of Applied Earth Observation and Geoinformation, 56*, 44-53. doi:https://doi.org/10.1016/j.jag.2016.11.005

Robinson, D., Hayes, A., & Couch, S. (2022). broom: Convert Statistical Objects into Tidy Tibbles. Retrieved from https://CRAN.R-project.org/package=broom

Rudgers, J. A., Kivlin, S. N., Whitney, K. D., Price, M. V., Waser, N. M., & Harte, J. (2014). Responses of high-altitude graminoids and soil fungi to 20 years of experimental warming. *Ecology (Durham), 95*(7), 1918-1928. doi:10.1890/13-1454.1

Ruotsalainen, A. L., & Kytöviita, M.-M. (2004). Mycorrhiza Does Not Alter Low Temperature Impact on Gnaphalium norvegicum. *Oecologia, 140*(2), 226-233. doi:10.1007/s00442-004-1586-3

Ruotsalainen, A. L., Tuomi, J., & Väre, H. (2002). A model for optimal mycorrhizal colonization along altitudinal gradients. *Silva fennica (Helsinki, Finland : 1967), 36*(3), 681-694. doi:10.14214/sf.533

Ruotsalainen, A. L., Väre, H., Oksanen, J., & Tuomi, J. (2004). Root Fungus Colonization along an Altitudinal Gradient in North Norway. *Arctic, Antarctic, and Alpine Research, 36*(2), 239-243. doi:10.1657/1523-0430(2004)036[0239:RFCAAA]2.0.CO2

Schmidt, I. K., Jonasson, S., & Michelsen, A. (1999). Mineralization and microbial immobilization of N and P in arctic soils in relation to season, temperature and nutrient amendment. *Applied soil ecology : a section of Agriculture, ecosystems & environment, 11*(2), 147-160. doi:10.1016/S0929-1393(98)00147-4

Schmidt, I. K., Jonasson, S., Shaver, G. R., Michelsen, A., & Nordin, A. (2002). Mineralization and distribution of nutrients in plants and microbes in four arctic ecosystems: responses to warming. *Plant and soil, 242*(1), 93-106. doi:10.1023/A:1019642007929

Schüßler, A., Schwarzott, D., & Walker, C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological research, 105*(12), 1413-1421. doi:10.1017/S0953756201005196

Semenchuk, P. R., Gillespie, M. A. K., Rumpf, S. B., Baggesen, N., Elberling, B., & Cooper, E. J. (2016). High Arctic plant phenology is determined by snowmelt patterns but duration of phenological periods is fixed: an example of periodicity. *Environmental research letters, 11*(12), 125006. doi:10.1088/1748-9326/11/12/125006

Shaver, G. R., Canadell, J., Chapin, F. S., Gurevitch, J., Harte, J., Henry, G., . . . Rustad, L. (2000). Global Warming and Terrestrial Ecosystems: A Conceptual Framework for Analysis. *BioScience, 50*(10), 871-882. doi:10.1641/0006-3568(2000)050[0871:GWATEA]2.0.CO2

Smith, L. C., Sheng, Y., MacDonald, G. M., & Hinzman, L. D. (2005). Disappearing Arctic Lakes. *Science, 308*(5727), 1429. Retrieved from https://www.proquest.com/scholarly-journals/disappearing-arctic-lakes/docview/213619408/se-2?accountid=11162

Soudzilovskaia, N. A., van der Heijden, M. G. A., Cornelissen, J. H. C., Makarov, M. I., Onipchenko, V. G., Maslov, M. N., . . . Plant Microbe, I. (2015). Quantitative assessment of the differential impacts of arbuscular and ectomycorrhiza on soil carbon cycling. *The New phytologist, 208*(1), 280-293. doi:10.1111/nph.13447

Tarnocai, C., Canadell, J. G., Schuur, E. A. G., Kuhry, P., Mazhitova, G., & Zimov, S. (2009). Soil organic carbon pools in the northern circumpolar permafrost region. *Global biogeochemical cycles, 23*(2).

Trouvelot, A., Kough, J. L., & Gianinazzi-Pearson, V. (1986). Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une signification fonctionnelle. In (pp. 217-221): Paris : Institut national de le recherche agronomique, c1986.

van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist, 205*(4), 1406-1423. doi:https://doi.org/10.1111/nph.13288

Vare, H., Vestberg, M., & Ohtonen, R. (1997). Shifts in mycorrhiza and microbial activity along an oroarctic altitudinal gradient in Northern Fennoscandia. *Arctic and alpine research, 29*(1), 93-104. doi:10.2307/1551839

Varma, A., Prasad, R., & Tuteja, N. (2017). *Mycorrhiza - Function, Diversity, State of the Art*. Cham: Cham: Springer International Publishing AG.

Vierheilig, H., Coughlan, A. P., Wyss, U., & Piché, Y. (1998). Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi. *Applied and Environmental Microbiology, 64*(12), 5004-5007. doi:10.1128/aem.64.12.5004-5007.1998

Vierheilig, H., Schweiger, P., & Brundrett, M. (2005). An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots†. *Physiologia Plantarum, 125*(4), 393-404. doi:https://doi.org/10.1111/j.1399-3054.2005.00564.x

Väre, H., Vestberg, M., & Eurola, S. (1992). Mycorrhiza and root-associated fungi in Spitsbergen. *Mycorrhiza, 1*(3), 93-104. doi:10.1007/BF00203256

Wang, B., Funakoshi, D., Dalpé, Y., & Hamel, C. (2002). Phosphorus-32 absorption and translocation to host plants by arbuscular mycorrhizal fungi at low root-zone temperature. *Mycorrhiza, 12*(2), 93-96. doi:10.1007/s00572-001-0150-9

Wehner, J., Antunes, P. M., Powell, J. R., Mazukatow, J., & Rillig, M. C. (2010). Plant pathogen protection by arbuscular mycorrhizas: A role for fungal diversity? *Pedobiologia, 53*(3), 197-201. doi:https://doi.org/10.1016/j.pedobi.2009.10.002

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*: Springer-Verlag New York.

Wickham, H., François, R., Henry, L., & Müller, K. (2022). dplyr: A Grammar of Data Manipulation. Retrieved from https://CRAN.R-project.org/package=dplyr

Wickham, H., & Seidel, D. (2022). scales: Scale Functions for Visualization. Retrieved from https://CRAN.R-project.org/package=scales

Wild, J., Kopecký, M., Macek, M., Šanda, M., Jankovec, J., & Haase, T. (2019). Climate at ecologically relevant scales: A new temperature and soil moisture logger for long-term microclimate measurement. *Agricultural and forest meteorology, 268*, 40-47. doi:10.1016/j.agrformet.2018.12.018

Wilke, C. (2020). cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. Retrieved from https://CRAN.R-project.org/package=cowplot

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J. M., . . . Zobel, M. (2010). The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *The New phytologist, 188*(1), 223-241. doi:10.1111/j.1469-8137.2010.03334.x

Öpik, M., Zobel, M., Cantero, J. J., Davison, J., Facelli, J. M., Hiiesalu, I., . . . Moora, M. (2013). Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza, 23*(5), 411-430. doi:10.1007/s00572-013-0482-2

Appendix A

Popular science summary

Arctic grasses and fungi team up in response to climate change

Our planet is warming, and the warming is most extreme in the Arctic, where temperatures are increasing three times faster than the global average. It is estimated that the Arctic stores up to half of earths terrestrially bound carbon in its cold soils. Carbon that if decomposed becomes greenhouse gasses that exacerbate climate change further and impact future life on earth. Because of this, understanding how growth and decomposition are affected by increasing temperatures is of utmost importance.

Both satellite data and field studies show that the Arctic is greening. Plants are growing bigger and areas previously uninhabited by plants are being colonized. So far, grasses and shrubs seem to be the plants benefitting most from the changing climate, whereas it is disadvantageous for lichens and mosses as they are not able to compete with the expanding plant growth. However, changes are not only occurring above ground. Below the surface, the soil ecosystem is also adapting to a warmer climate. In the cold environment of the Arctic, microbial organisms decompose dead organic matter slowly and the nutrients bound in it remains locked away from plants. However, as Arctic soils warm, fungal partners more common in warmer ecosystems have the potential to help plants with their nutrient uptake. These fungi, called arbuscular mycorrhizal fungi, that use their hyphae (fine network root-like growth) to take up nutrients plants cannot reach and transports them to plants. In exchange for supplying plants with nutrients the fungi receive sugars from its plant partner. This symbiosis is uncommon in the Arctic today but could become more common as the climate changes. As some Arctic grasses team up with fungi they could gain an advantage over other plants, potentially explaining why grasses are becoming more common in the Arctic.

This study aims to find how warmer temperatures affect the extent of grass – fungi symbiosis, and to what extent that effects the nutrient content of the plant. The study was done in Swedish Lapland, at the Latnjajaure field station located ~1000 meters above sea level by collecting the Arctic grass *Poa alpina* and soil samples at 12 sites with varying temperatures. The amount of fungal symbiosis formed in the roots of *P. alpina* was measured and compared to the nutrient content of soil samples and the grass shoots. By comparing the results from warmer and cooler sites, this study aims to answer what the future role of arbuscular mycorrhizal symbiosis could be in a warmer Arctic climate.

This study was found a likely positive relationship between increasing temperatures and increasing fungal symbiosis

Appendix B

Table 3. The results from three cummulative ordinal regression model testing the relationship between mycorrhizal coloniation and below ground temperature (BGT), ground surface temperature (GST), above ground temperature (AGT), soil N, and soil P and interactions between the independent variables.

Variable	Lower 95% confidence intervall	Regression coefficiant	Upper 95% confidence intervall
BGT	-1,08	-0,03	1,11
Soil N	-1,45	-0,39	0,70
Soil P	-2,35	-0,67	1,00
Soil N x Soil P	-1,62	-0,09	1,38
Soil N x BGT	-1,45	1.00	3,34
Soil P x BGT	-2,44	0,15	2,66
Soil P x Soil N x BGT	-5,06	-0,85	3,37
GST	-0,34	0,72	1,78
Soil N	-4,45	-0,12	4,25
Soil P	-3,09	0,56	4,28
Soil N x Soil P	-2,95	0,82	4,33
Soil N x GST	-0,32	1,08	2,44
Soil P x GST	-0,57	0,80	2,29
Soil P x Soil N x GST	-5,31	0,63	6,63
AGT	-1,24	0,41	2,10
Soil N	-2,65	0,30	3,12
Soil P	-1,83	0,69	3,16
Soil N x Soil P	-4,09	-0,03	4,25
Soil N x AGT	-1,15	0,73	2,80
Soil P x AGT	-2,03	0,16	2,51
Soil P x Soil N x AGT	-2,35	1,10	4,70

Table 4. The results from six linear regression analyses testing the relationship between soil N% and soil P% (both presented in percent dry mass) and below ground temperature (BGT), ground surface temperature (GST) and above ground temperature (AGT). No significant relationships were found, but potential negative trends were found between soil P% and GST and AGT.

Relationship	Slope	p value	Adjusted R^2
Soil N x BGT	-0,00013	0,77	0,09
Soil N x GST	0,00008	0,85	-0,10
Soil N x AGT	0,00028	0,52	-0,05
Soil P x BGT	-0,00004	0,99	-0.10
Soil P x GST	-0,00448	0,13	0,13
Soil P x AGT	-0,00520	0,07	0,21

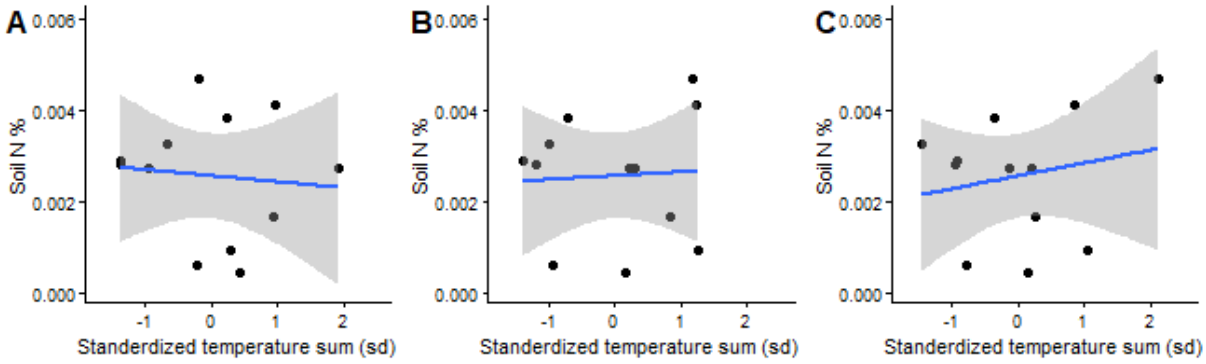

Figure 7. Linear regression analysis of relationship between plot temperature (standardized and varying in standard deviations around the mean) and soil N. **A** The relationship between soil N and below ground temperature. No significant relationship was found (p: 0,77) **B** The relationship between soil N and ground surface temperature. No significant relationship was found (p: 0,85). **C** The relationship between soil N% and above ground temperature. No significant relationship was found (p: 0,52)

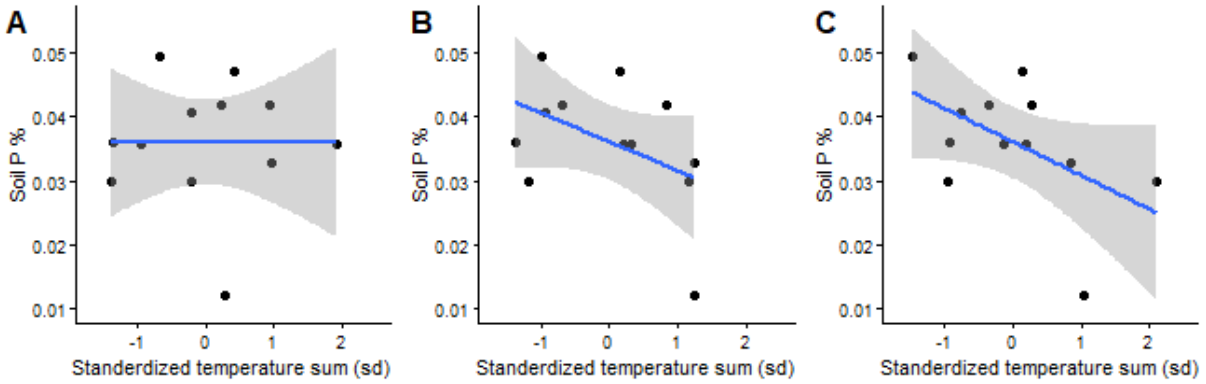

Figure 8. Linear regression analysis of relationship between plot temperature (standardized and varying in standard deviations around the mean) and soil P. **A** The relationship between soil P and below ground temperature. No significant relationship was found (p: 0,99) **B** The relationship between soil P and ground surface temperature. A potentiall negative trend between temperature and soil P was found (p: 0,13, slope: -0,00448 and R^2: 0,13). **C** The relationship between soil P and above ground temperature. A negative trend was found (p: 0,07, slope: 0,00520 and R^2: 0,21).

Appendix C

Ordinal logistic regression R script

```
library(devtools)
library(cmdstanr)
library(tidyverse)
library(ggplot2)
library(brms)
library(dplyr)
library(cowplot)
library(scales)
library(RColorBrewer)
setwd("C:/Users/Otto Minas/Documents/GU/Masterexamen/Method")
R <- readxl::read_xlsx("Data/R MycCol/R.xlsx")
# Formatting data
R <- R%>% mutate_at(7:26, as.integer)
R <- R%>%
  select(-`M%`)%>%
  pivot_longer(6:25,
               names_to = "Rotbit",
               values_to = "Koloniseringsgrad")
R%>% print(n=200)
R <- R%>% na.omit
# Change names
names(R)[1:5] <- c("tminus8cm","t0cm","t15cm","plotid","sampleid")
# Change ranges of colonization classes
R$Koloniseringsgrad <- R$Koloniseringsgrad + 1
R%>% ggplot(aes(tminus8cm,Koloniseringsgrad)) + geom_point(alpha=0.1)
gc()
# Reducing size of temp variable values ((x - mean) / standard deviation)
R$stdtemp8 <- R$tminus8cm
R$stdtemp8 <- R$stdtemp8 - mean(R$stdtemp8)
R$stdtemp8 <- R$stdtemp8/sd(R$stdtemp8)
R$stdtemp0 <- R$t0cm
R$stdtemp0 <- R$stdtemp0 - mean(R$stdtemp0)
R$stdtemp0 <- R$stdtemp0/sd(R$stdtemp0)
R$stdtemp15 <- R$t15cm
R$stdtemp15 <- R$stdtemp15 - mean(R$stdtemp15)
R$stdtemp15 <- R$stdtemp15/sd(R$stdtemp15)
# Run OLR BGT
fit_sc2 <- brm(
  formula = Koloniseringsgrad ~ 1 + (stdtemp8) + (1|plotid:sampleid)+ (1|plotid),
  data = R,
  family = cumulative("probit"),
  cores = 8,
  backend = "cmdstanr",
  chains = 8,
  iter = 1000,
  control = list(adapt_delta=0.9)
)
pp_check(fit_sc2,type = "bars")
R[R$stdtemp8 > 0,]
summary(fit_sc2)
plot(fit_sc2)
h_temp8<-hypothesis(fit_sc2,"stdtemp8>0")
hypothesis(fit_sc2,"stdtemp8=0")
h_temp8
plot(h_temp8)
# Plot OLR BGT
```

```
conditional_effects(fit_sc2,categorical = TRUE,prob = 0.95)

BGTemp <- conditional_effects(fit_sc2,categorical = TRUE,prob = 0.95)

# Check if the model works similarly for low and high temperatures

pp_check(fit_sc2,type = "bars", newdata=R[R$stdtemp15 > 0,], ndraws=100)

pp_check(fit_sc2,type = "bars", newdata=R[R$stdtemp15 < 0,], ndraws=100)

# Run OLR SST

fit_sc2.0 <- brm(

  formula = Koloniseringsgrad ~ 1 + (stdtemp0) + (1|plotid:sampleid)+ (1|plotid),

  data = R,

  family = cumulative("probit"),

  cores = 8,

  backend = "cmdstanr",

  chains = 8,

  iter = 1000,

  control = list(adapt_delta=0.9)

)

pp_check(fit_sc2.0,type = "bars")

summary(fit_sc2.0)

plot(fit_sc2.0)

h_temp0<- hypothesis(fit_sc2.0,"stdtemp0>0")

hypothesis(fit_sc2.0,"stdtemp0=0")

h_temp0

plot(h_temp0)

# Plot GST

conditional_effects(fit_sc2.0,categorical = TRUE,prob = 0.95)

GSTtemp <- conditional_effects(fit_sc2.0,categorical = TRUE,prob = 0.95)

#Check if the model works similarly for low and high temperatures

pp_check(fit_sc2.0,type = "bars", newdata=R[R$stdtemp0 > 0,], ndraws=100)

pp_check(fit_sc2.0,type = "bars", newdata=R[R$stdtemp0 < 0,], ndraws=100)

# Run OLR15 OLR

fit_sc2.15 <- brm(

  formula = Koloniseringsgrad ~ 1 + (stdtemp15) + (1|plotid:sampleid)+ (1|plotid),

  data = R,

  family = cumulative("probit"),

  cores = 8,

  backend = "cmdstanr",

  chains = 8,

  iter = 1000,

  control = list(adapt_delta=0.9)

)

pp_check(fit_sc2.15,type = "bars")

R[R$stdtemp15 > 0,]

summary(fit_sc2.15)

plot(fit_sc2.15)

h_temp15<- hypothesis(fit_sc2.15,"stdtemp15>0")

hypothesis(fit_sc2.15,"stdtemp15=0")

h_temp15

plot(h_temp15)

#Plot AGT

conditional_effects(fit_sc2.15,categorical = TRUE,prob = 0.95)

AGTtemp <- conditional_effects(fit_sc2.15,categorical = TRUE,prob = 0.95)

#Check if the model works similarly for low and high temperatures

pp_check(fit_sc2.15,type = "bars", newdata=R[R$stdtemp15 > 0,], ndraws=100)
```

```
pp_check(fit_sc2.15,type = "bars",
newdata=R[R$stdtemp15 < 0,],
ndraws=100)

#Validating models

loo_sc2<- loo(fit_sc2)

loo_sc0<- loo(fit_sc2.0)

loo_sc15<- loo(fit_sc2.15)

loo_compare(loo_sc2,loo_sc0)

pp_check(fit_sc2,type = "bars")

R[R$stdtemp8 > 0,]

summary(fit_sc2)

plot(fit_sc2)

h_temp8<-hypothesis(fit_sc2,"stdtemp8>0")

h_temp8

plot(h_temp8)

#BGT

plot(BGTemp8,plot = FALSE)[[1]] +

labs(x = "Standardized temperature sum
(sd)",

y = "Probability",

title = "") +

theme(legend.position = "none")+

scale_x_continuous(limits = c(-1.5,2.0))+

scale_color_brewer(palette = 3, type =
"qual", labels = c("0", "1", "2","3", "4", "5"),
name="Mycorrhizal
colonization\ncategory")+

scale_fill_brewer(palette = 3, type = "qual",
labels = c("0", "1", "2","3", "4", "5"),
name="Mycorrhizal
colonization\ncategory")

#GST

plot(SSTtemp,plot = FALSE)[[1]] +

labs(x = "Standardized temperature sum
(sd)",

y = "Probability",

title = "") +

theme(legend.position = "none")+

scale_x_continuous(limits = c(-1.5,2.0))+

scale_color_brewer(palette = 3, type =
"qual", labels = c("0", "1", "2","3", "4", "5"),
name="Mycorrhizal
colonization\ncategory")+

scale_fill_brewer(palette = 3, type = "qual",
labels = c("0", "1", "2","3", "4", "5"),
name="Mycorrhizal
colonization\ncategory")

#AGT

plot(AGTtemp,plot = FALSE)[[1]] +

labs(x = "Standardized temperature sum
(sd)",

y = "Probability",

title = "") +

theme(legend.position = "right")+

guides(color=guide_legend(nrow=3,
byrow=TRUE))+

scale_x_continuous(limits = c(-1.5,2.0))+

scale_color_brewer(palette = 3, type =
"qual", labels = c("0", "1", "2","3", "4", "5"),
name="Mycorrhizal\ncolonization
category")+

scale_fill_brewer(palette = 3, type = "qual",
labels = c("0", "1", "2","3", "4", "5"),
name="Mycorrhizal\ncolonization
category")

#Plotting spread of data

plot8 <-ggplot(R)+

geom_point(aes(x=stdtemp8,
y=Koloniseringsgrad,
colour=factor(Koloniseringsgrad)),
position="jitter", alpha = 0.2)+

scale_color_brewer(palette = 3, type =
"qual", name="Mycorrhizal
colonization\ncategory")+

scale_fill_brewer(palette = 3, type = "qual",
name="Mycorrhizal
colonization\ncategory")+

geom_violin(aes(x=stdtemp8,
y=Koloniseringsgrad, group=stdtemp15,
fill=NA),

position=position_dodge(width = 1),
width=0.25)+
```

```
theme_bw()+
labs(x="Standardized temperature sum (sd)",
y = "Mycorrhizal\ncolonization category",
title = "",
color="Mycorrhizal
colonization\ncategory")+
scale_y_continuous(breaks =
c(0,1,2,3,4,5))+
scale_x_continuous(limits = c(-1.7,2.5))+
theme(legend.position = "none")+
theme(text = element_text(size = 8))
plot0 <-ggplot(R)+
geom_point(aes(x=stdtemp0,
y=Koloniseringsgrad,
colour=factor(Koloniseringsgrad)),
position="jitter", alpha = 0.2)+
scale_color_brewer(palette = 3, type =
"qual", name="Mycorrhizal
colonization\ncategory")+
scale_fill_brewer(palette = 3, type = "qual",
name="Mycorrhizal
colonization\ncategory")+
geom_violin(aes(x=stdtemp0,
y=Koloniseringsgrad, group=stdtemp15,
fill=NA),
position=position_dodge(width = 1),
width=0.25)+
theme_bw()+
labs(x="Standardized temperature sum (sd)",
y = "Mycorrhizal\ncolonization category",
title = "",
color="Mycorrhizal
colonization\ncategory")+
scale_y_continuous(breaks =
c(0,1,2,3,4,5))+
scale_x_continuous(limits = c(-1.7,2.5))+
theme(legend.position = "none")+
theme(text = element_text(size = 8))
plot15 <-ggplot(R)+
geom_point(aes(x=stdtemp15,
y=Koloniseringsgrad,
colour=factor(Koloniseringsgrad)),
position="jitter", alpha = 0.2)+
scale_color_brewer(palette = 3, type =
"qual", name="Mycorrhizal
colonization\ncategory")+
scale_fill_brewer(palette = 3, type = "qual",
name="Mycorrhizal
colonization\ncategory")+
geom_violin(aes(x=stdtemp15,
y=Koloniseringsgrad, group=stdtemp15,
fill=NA),
position=position_dodge(width = 1),
width=0.25)+
theme_bw()+
labs(x="Standardized temperature sum (sd)",
y = "Mycorrhizal\ncolonization category",
title = "",
color="Mycorrhizal
colonization\ncategory")+
scale_y_continuous(breaks =
c(0,1,2,3,4,5))+
scale_x_continuous(limits = c(-1.7,2.5))+
theme(legend.position = "none")+
theme(text = element_text(size = 8))
#extract ledgend
legend <- get_legend(plot8 +
theme(legend.box.margin = margin(0, 0, 0,
12))+
theme(legend.position = "bottom")+
theme(legend.key.height= unit(0.4, 'cm'),
legend.key.width= unit(1, 'cm')))
plot_grid(plot8, plot0, plot15, legend,
rel_heights = c(5,5,5,1), nrow = 4, labels =
c('A', 'B', 'C'), label_size = 12)
```

Regression analysis R script

```
setwd("C:/Users/Otto Minas/Documents/GU/Masterexamen/Method/data/R Nut")
R <- readxl::read_xlsx("R.nutrients.xlsx")
install.packages("ggplot2")
install.packages("dplyr")
install.packages("broom")
install.packages("ggpubr")
library(ggplot2)
library(dplyr)
library(broom)
library(ggpubr)
summary(R)
#Check for normal dist
hist(R$`soil P%`)
hist(R$`soil N%`)
hist(R$`tissue N%`)
hist(R$`tissue P%`)
#Transform soil P
TsP <-sqrt(max(R$`soil P%`+1) - R$`soil P%`)
hist(TsP)
#Transform soil N
TsN <- sqrt(R$`soil N%`)
hist(TsN)
## Not successfull (do a nonparametric test)
# Test for normality with sharpio wilks
library(rstatix)
shapiro_test(R$`soil P%`)
shapiro_test(R$`soil N%`)
shapiro_test(R$`tissue N%`)
shapiro_test(R$`tissue P%`)
#Change names and normalize temp
names(R)[2:4] <- c("tminus8cm","t0cm","t15cm")
#-8 cm
R$stdtemp8 <- R$tminus8cm
R$stdtemp8 <- R$stdtemp8 - mean(R$stdtemp8)
R$stdtemp8 <- R$stdtemp8/sd(R$stdtemp8)
#0 cm
R$stdtemp0 <- R$t0cm
R$stdtemp0 <- R$stdtemp0 - mean(R$stdtemp0)
R$stdtemp0 <- R$stdtemp0/sd(R$stdtemp0)
#15 cm
R$stdtemp15 <- R$t15cm
R$stdtemp15 <- R$stdtemp15 - mean(R$stdtemp15)
R$stdtemp15 <- R$stdtemp15/sd(R$stdtemp15)
#Test for linearity TSP
plot(TsP ~ stdtemp8, data = R)
plot(TsP ~ stdtemp0, data = R)
plot(TsP ~ stdtemp15, data = R)
#Test for linearity tissue N
plot(R$`tissue N%` ~ stdtemp8, data = R)
plot(R$`tissue N%` ~ stdtemp0, data = R)
plot(R$`tissue N%` ~ stdtemp15, data = R)
#Testing regression for TsP -8cm
stdtemp8.TsP.lm <- lm(TsP ~ stdtemp8, data = R)
summary(stdtemp8.TsP.lm)
#Testing regression for TsP 0cm
stdtemp0.TsP.lm <- lm(TsP ~ stdtemp0, data = R)
summary(stdtemp0.TsP.lm)
#Testing regression for TsP 15cm
stdtemp15.TsP.lm <- lm(TsP ~ stdtemp15, data = R)
summary(stdtemp15.TsP.lm)
#Testing regression for tissue N -8cm
stdtemp8.tissueN.lm <- lm(R$`tissue N%` ~ stdtemp8, data = R)
summary(stdtemp8.tissueN.lm)
```

```
#Testing regression for tissue N 0cm
stdtemp0.tissueN.lm <- lm(R$`tissue N%` ~ stdtemp0, data = R)
summary(stdtemp0.tissueN.lm)
#Testing regression for tissue N 15cm
stdtemp15.tissueN.lm <- lm(R$`tissue N%` ~ stdtemp15, data = R)
summary(stdtemp15.tissueN.lm)
#Testing regression for soil N -8cm
stdtemp8.soilN.lm <- lm(R$`soil N%` ~ stdtemp8, data = R)
summary(stdtemp8.tissueN.lm)
#Testing regression for soil N 0cm
stdtemp0.soilN.lm <- lm(R$`soil N%` ~ stdtemp0, data = R)
summary(stdtemp0.tissueN.lm)
#Testing regression for soil N 15cm
stdtemp15.soilN.lm <- lm(R$`soil N%` ~ stdtemp15, data = R)
summary(stdtemp15.tissueN.lm)
#Test all for homogeneity of variance
par(mfrow=c(2,2))
#TSP
plot(stdtemp8.TsP.lm)
plot(stdtemp0.TsP.lm)
plot(stdtemp15.TsP.lm)
#Tissue N
plot(stdtemp8.tissueN.lm)
plot(stdtemp0.tissueN.lm)
plot(stdtemp15.tissueN.lm)
#Tissue P
plot(stdtemp8.soilN.lm)
plot(stdtemp0.soilN.lm)
plot(stdtemp15.soilN.lm)
par(mfrow=c(1,1))
#Parametric analyses deemed not suitable
#Spearman rank
# Testing SR for soil P 8 cm
stdtemp8.soilp.sr<- cor.test(R$stdtemp8, R$`soil P%`, method = 'spearman')
stdtemp8.soilp.sr
# Testing SR for soil P 0 cm
stdtemp0.soilp.sr<- cor.test(R$stdtemp0, R$`soil P%`, method = 'spearman')
stdtemp0.soilp.sr
# Testing SR for soil P 15 cm
stdtemp15.soilp.sr<- cor.test(R$stdtemp15, R$`soil P%`, method = 'spearman')
stdtemp15.soilp.sr
# Testing SR for soil N 8 cm
stdtemp8.soiln.sr<- cor.test(R$stdtemp8, R$`soil N%`, method = 'spearman')
stdtemp8.soiln.sr
# Testing SR for soil N 0 cm
stdtemp0.soiln.sr<- cor.test(R$stdtemp0, R$`soil N%`, method = 'spearman')
stdtemp0.soiln.sr
# Testing SR for soil N 15 cm
stdtemp15.soiln.sr<- cor.test(R$stdtemp15, R$`soil N%`, method = 'spearman')
stdtemp15.soiln.sr
# Testing SR for tissue N 8 cm
stdtemp8.tissuen.sr<- cor.test(R$stdtemp8, R$`tissue N%`, method = 'spearman')
stdtemp8.tissuen.sr
# Testing SR for tissue N 0 cm
stdtemp0.tissuen.sr<- cor.test(R$stdtemp0, R$`tissue N%`, method = 'spearman')
stdtemp0.tissuen.sr
# Testing SR for tissue N 15 cm
stdtemp15.tissuen.sr<- cor.test(R$stdtemp15, R$`tissue N%`, method = 'spearman')
stdtemp15.tissuen.sr
# Testing SR for tissue P 8 cm
```

```
stdtemp8.tissuep.sr<- cor.test(R$stdtemp8, R$`tissue P%`, method = 'spearman')

stdtemp8.tissuep.sr

# Testing SR for tissue P 0 cm

stdtemp0.tissuep.sr<- cor.test(R$stdtemp0, R$`tissue P%`, method = 'spearman')

stdtemp0.tissuep.sr

# Testing SR for tissue P 15 cm

stdtemp15.tissuep.sr<- cor.test(R$stdtemp15, R$`tissue P%`, method = 'spearman')

stdtemp15.tissuep.sr

#Plot

R%>%
  ggplot(aes(stdtemp8,`tissue P%`)) +
  geom_point()+
  stat_smooth(method="lm")
```