Arbuscular mycorrhizal fungal communities and root colonisation differ in relation to plant host and soil nutrients

Arbuskul soppasamfeløg og soppprótagróður í planturótum broytist í mun til plantuslag og taðevni í moldini

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Abstract

Field samples from two coexisting plant species were examined to see whether they were colonised by distinct fungal communities, and whether the colonisation pattern differed between the two plant species.

Two plant species, *Agrostis capillaris* and *Ranunculus acris* from four mountain slopes in the Faroe Islands were examined for percentage root length colonisation (%RLC) including the amount of arbuscules and vesicles, and the arbuscular mycorrhizal (AM) fungal types in the roots identified by cloning and sequencing.

Fewer AM fungal types were found in *Ranunculus acris* than in *Agrostis capillaris,* but the %RLC was greater in *Ranunculus acris*, which also showed seasonal variability. Vesicles were more abundant in *Ranunculus acris.*

Statistical analysis suggested that the AM fungal communities colonising the two plant species were distinct. The root colonisation in *Ranunculus acris* responded to most soil nutrients, but in particular to phosphate and nitrogen. The root colonisation in *Agrostis capillaris* responded most to nitrogen.

In conclusion the data support the hypothesis that plants from a single habitat that differ in mycorrhizal dependency would be colonised by distinct fungal communities. The fungal community is probably a reflection of plant community, nutrient availability, temperature, as well as other biotic and abiotic factors.

Úrtak

Sýni frá tveimum plantusløgum, sum vaksa saman, vórðu kannað fyri at vita, um tey hýsa ymisk soppasamfeløg, og um soppagróðurin vísti ymisk mynstur í teimum báðum plantusløgunum.

Tvey plantusløg, vanligt fínagras *(Agrostis*

capillaris) og svínasólja (*Ranunculus acris)* frá fýra fjallasíðum í Føroyum vórðu kannað fyri prosentvísu rótlongdina við sopprót (%RLS). Talið av arbusklum og vesiklum vórðu eisini skrásett, og arbuskul sopparnir vóru eyðmerktir við tøkni sum nýtir kloning og sekvensering.

Færri sløg av arbuskul soppum vóru funnin í svínasólju enn í vanligum fínagrasi, men meira %RLS var í rótum hjá svínasólju, og her var eisini árstíðarmunur. Vesiklar vóru fleiri í svínasólju.

Hagfrøðiligar greiningar benda á, at soppasamfeløgini í rótunum á teimum báðum plantusløgunum vóru ymisk. Í svínasólju var samband millum nøgdina av %RLS og flestu taðevni í moldini, tó serliga fosfat og nitrogen, meðan í vanligum fínagrasi var tað serliga nitrogen sum hevði týdning.

Niðurstøðan er, at dáturnar stuðla tilgitingini, at plantur frá sama búøki, har planturnar hava ymiskan tørv á sopprót, hava sopprót við øðrvísi soppasløgum. Soppasamansetingin er helst ein avmyndan av plantusamfelagnum, hvørji taðevni eru tøk, hita, eins og bæði lívrunnum og ólívrunnum viðurskiftum.

Abbrevations

%FR	percent fine roots
%RLC	percent root length colonisation
%RLS	prosentvís rótlongd við sopprót
AM	arbuscular mycorrhiza(l)
LOI	loss of ignition
N	nitrogen
P	phosphate

Introduction

Mycorrhizas are beneficial symbioses between fungi and plants. Until recently, the arbuscular mycorrhizal (AM) fungal symbiosis has been considered as non-specific in relation to plant host. Smith and Read (1997) state that an AM fungus isolated from one species of host plant will colonise any other species that has been shown to be capable of forming arbuscular mycorrhiza, and that this lack of specificity has important consequences for ecological interactions in plant communities, allowing individual fungi to colonise a broad range of host plants. Even though Smith and Read (1997) consider the symbiosis to be non-specific they acknowledge the fact that there are differences in the extent to which species of plants become colonised by mycorrhizal fungi. Thus, the term selectivity rather than specificity for the plant-fungal interaction has been suggested (Helgason *et al.*, 2002). Smith and Read (2008) reviewed several studies and concluded that specificity or selectivity in AM symbioses may well be somewhat higher than previously perceived.

Studies have shown that an AM fungal community can consist of a considerable number of fungal types, and that a plant community with high plant species diversity also supports a rich AM fungal community. For example Bever *et al.* (2001) examined the AM fungal spores in a one-hectare field with approximately 50 plant species and identified 37 different species of AM fungi. The high number of fungal species together with the high plant diversity found by Bever and co-workers (2001) could be another indicator of an associa-

tion between fungal diversity and plant diversity.

Although approximately 150 AM fungal species have been described, it is increasingly apparent that there are numerous other species of fungi in natural ecosystems, and that many of these are highly selective as to the host they will colonise (Fitter *et al.*, 2004). Support of this view is work using molecular methods to study AM fungal diversity in natural ecosystems, which all found different AM fungal communities in different plant hosts (e.g. Husband *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002, 2003; Öpik *et al.*, 2003). All the aforementioned studies examined more than one plant species, and found a difference in the AM fungal commnities in the different plant species, or site-dependent differences. Additionally, they found both clearly widespread taxa as well as new taxa.

The current view on the biology of the AM fungi is based on culturable fungi, and it is likely that the fungi in the field are different from the small subset of easily cultured species, of which the latter might turn out to be generalists (Fitter, 2005).

Plants differ in their mycorrhiza-dependency (Fitter and Peat, 1994). One "rule of thumb" is that plants with coarse roots are dependent on mycorrhiza for nutrient uptake (Merryweather and Fitter, 1996). In contrast, plants with fine roots are less dependent on mycorrhiza for nutrient uptake, but might benefit for other reasons, such as pathogen defence (Newsham *et al.*, 1995a). Those findings led to the hypothesis of multi-functionality and biodiversity in AM (Newsham *et al.*, 1995b). The hypothesis states that different AM fungi have different functions: some might be good for nutrient uptake, others for protection against pathogens, and still others for drought, just to mention a few examples. Support for the multifunctionality and biodiversity hypothesis comes from the work of van der Heijden *et al.* (1998), who found that with more AM fungal taxa the plant biodiversity, plant biomass and plant phosphate content all increased.

It is almost certain, that AM fungi differ in their functional attributes, and as such the diversity of fungi in a community should have ecological consequences, including consequences for the cycling of nitrogen, phosphorus and carbon (Fitter, 2005). For example Johnson et al. (2003) demonstrated a positive correlation with AM fungal diversity and shoot phosphorus concentration, and Lekberg *et al.* (2007) found that the level of organic carbon and total nitrogen appeared to be important factors in shaping fungal communities. Moreover, work with culturable fungi has shown some functional complementarity with respect to phosphorus acquisition between AM fungal species (Jansa *et al.*, 2008).

Thus, one may expect that most plant communities support numerous species of AM fungi, which may be functionally distinct, and that these fungi also will show plant host selectivity.

The purpose of the present study was to determine whether the communities of AM fungi found in plants from a single habitat that differ in mycorrhizal dependency will be colonised by distinct fungal

communities. The working hypothesis was that the benefits different plants acquire from colonisation will differ according to soil nutrients and phenology, and therefore that plants from a single habitat that differ in mycorrhizal dependency would be colonised by distinct fungal communities.

Material and methods

The two plant species selected for investigation were *Agrostis capillaris* L. and *Ranunculus acris* L. The grass *Agrostis capillaris* is one of the most common plant species in the Faroes, found both at high and low altitude. It has a relatively fine root system. The dicot *Ranunculus acris* is also found at both low and high altitudes, although at high altitudes it is mainly *Ranunculus acris* var. *pumilus*. The root system is relatively coarse.

The samples were from four mountain slopes in the Faroes. Two of the slopes face south-west, Gráfelli and Mosarøkur, and two face north, Sornfelli and Ørvisfelli. The sites are all open grassland and range from temperate zone (south-facing low altitude) to alpine zone (all high altitude sites) (Fosaa, 2004). During the summer period (June-August) 2001 and 2002, three cores of soil with vegetation, approximately 5-6 cm wide and 8 cm deep, were taken from each site on each sampling day. Three altitudinal sites were used, one site at low altitude (50-120 m a.s.l.), one at 350 m a.s.l. and one at 600 m a.s.l. Samples were taken from each mountain once a month during the summer, weather conditions permitting, in total 213 cores. DNA to measure the AM fungal diversity was only extracted from the samples obtained in 2002. To ensure as equal samles as possible, the selected sample was chosen by the combination of vegetation: *Ranunculus acris* and *Agrostis capillaris* in the same core was preferred. *Agrostis capillaris* is ubiquitous, while *Ranunculus acris* is patchily distributed. Since the two plants came from the same core, the same fungi should have had the opportunity to colonise both plant species.

The data presented here consist of mycorrhizal colonisation data obtained by clearing and staining roots following the procedure described by Vierheilig *et al.* (1998). Percent root length colonisation (%RLC) was measured following McGonigle *et al.* (1990). One hundred intersections were examined and mycorrhizal structures recorded for each intersection. The mycorrhizal structures were external AM hyphae, entry point, hyphae connected to arbuscules, hyphae connected to vesicles, hyphae with pegs that are likely to be AM, arbuscules, arbuscular coils, fine endophytes, vesicles. If one, two or more mycorrhizal structures were found in one intersection, they accounted for 1% RLC. In addition to mycorrhizal structures, an estimate of whether the plant root at the intersection point was coarse or fine was recorded (percent fine roots, %FR). A typical fine root was between 0.1 and 0.15 mm diameter, but a diameter up to 0.20 mm could be considered as a fine root if the stele was poorly developed.

The molecular analyses used PCR, cloning, RFLP, and sequencing as described in Olsen and Fitter (2004). Only roots sampled on two sampling days in

2002 were used for molecular analysis, and only for low (100 m a.s.l.) and high altitude (600 m a.s.l.). Samples were taken from all 4 locations used in this study. The samples were pooled, resulting in 14 samples for molecular analyses for each plant species, in total 28 samples. The amplification success-rate was low: only 15 samples amplified; of these, 10 were from *Agrostis capillaris* and only 5 from *Ranunculus acris*.

Only RFLP types with several occurrences were sequenced. The number of clones used in this analysis was 137, 101 from *Agrostis capillaris* and 36 from *Ranunculus acris*.

Soil samples from the same sites, but from 2000, were analysed for Mg, Ca, Na, K, H+, P (Olsen-P), N (Kjeldahl-N), pH and loss of ignition (LOI), see Lawesson *et al.* (2003).

All statistical analyses were carried out using SPSS version 11.03 for Mac OS X. The samples were tested for normal distribution with the One-Sample Kologorov-Smirnov test. %RLC was transformed (square-root) to meet criteria of normality. One-way Anova with Bonferroni correction was used to analyse means. For arbuscules and vesicles normality criteria were not met and instead they were analysed using nonparametric Kruskal-Wallis H and Mann-Whitney U tests. Posthoc tests of the nonparametric tests used Mann-Whitney tests with Bonferroni adjustments (target p value/number of paired comparisons).

When linear stepwise regression was performed, all soil nutrients values, chemical parameters as well as %RLC and %FR were used. The entry/removal criteria for the stepwise regression was the probability of F (enter 0.05, removal 0.1).

As the frequency of the fungal types found in this study varied with plant species, altitude, and aspect, a hierarchical loglinear analysis was carried out. Log-linear modelling has previously been used with similar data (e.g. Helgason *et al.*, 1999). Log-linear modelling is an analogue to multiple regression, examines all possible interactions, and is a type of multi-way frequency analysis. A saturated model is generated with all the relevant factors included; such a saturated model by definition has the probability of 1. Thereafter, by using backward elimination, models with fewer factors are generated and the 0-hypothesis is that the new model does not differ significantly from the saturated model. A p-value below 0.05 indicates that a factor that changed the saturated model has been removed.

Results

Soil temperature and nutrients

The mean soil temperature at low altitude was 10.9±0.45°C in 2001 and 12.4±0.38°C in 2002, with a decrease of more than 3°C from low to high altitude, and the south-facing slopes 0.5-1°C warmer than the northfacing (Olsen, 2006). The amount of soil phosphate and soil nitrogen were measured in 2000 and are shown in Fig. 1. Soil nitrogen was on average 0.6±0.07% and is a measure of both ammonium and organic nitrogen. The humus content was relatively high. The loss of igniton was on average 25±2.5%.

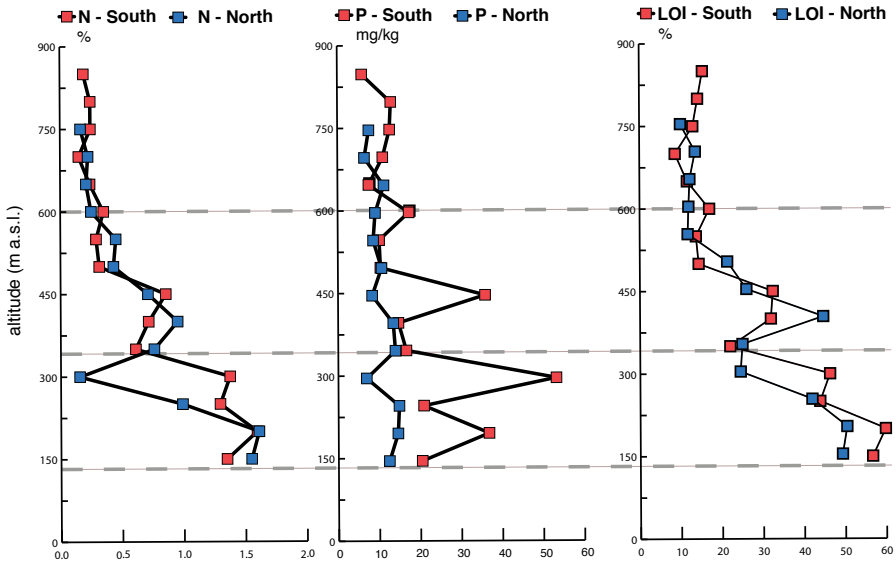

Fig. 1. Soil nitrogen (N), phosphate (P) and loss of ignition (LOI) along an altitudinal gradient. Values are means from the four slopes that were used in the fieldwork. The dashed horizontal lines at 600 m, 350 m and below 150 m indicate the altitudes where the samples were taken. Lines with red squares are means of the southfacing slopes, blue squares indicate means of the northfacing slopes. Soil nitrogen and LOI changed with altitude, with greater values ($p < 0.01$) at lover altitudes, while the amount of soil phosphate differed most with respect to aspect, with more available soil phosphate at southfacing slopes.

Root colonisation

The colonisation pattern in the two plants differed considerably. While the colonisation increased with altitude in *Agrostis capillaris*, it slightly (but non-significantly) decreased in *Ranunculus acris* (Fig. 2). There was a significant difference in the extent of colonisation between the two plant species, with *Ranunculus acris* averaging 31±1.9% and *Agrostis capillaris* 23±1.3% ($p < 0.01$).

There was a large difference in colonisation between the two sampling years with %RLC greater in 2001, which was colder than 2002, the colonisation in 2002 was only 40% of that in 2001 ($p < 0.001$).

The colonisation in *Agrostis capillaris* responded significantly to altitude ($p < 0.05$), but not to aspect. For both years the colonisation was greatest ($p < 0.01$) in roots from the highest altitude. However, analysed year by year this difference was only significant in 2002.

In *Ranunculus acris* the most profound response was to aspect, with greater colonisation at southfacing slopes ($p < 0.001$).

Factors that might account for differences in root colonisation in relation to samples and sites include soil nutrients and

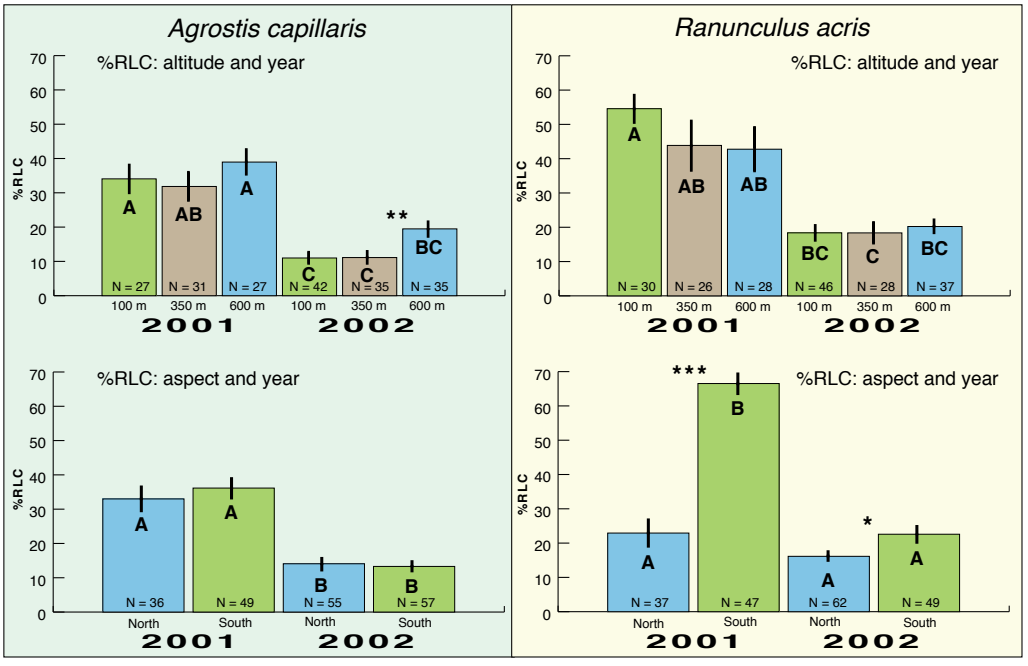

Fig. 2. The %RLC in *Agrostis capillaris* (left) and *Ranunculus acris* (right) at the three altitudes (above) investigated in this study, separated into the two sampling years. Values are means (n = 392) ±SE. Different letters indicate significantly different means. Stars indicate a difference found in a specific year only. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

plant characteristics, the latter are here represented by the percentage fraction of fine roots (%FR) with on average 55±1.1% fine roots in *Agrostis capillaris*, and 34±1.8% in *Ranunculus acris* (Fig. 3). In *Agrostis capillaris* the %FR was greater at high altitude ($p < 0.001$), but in *Ranunculus acris* high and low altitude had a similar amount of fine roots, but the roots were slightly coarser at the intermediate site at 350 m ($p < 0.05$). Aspect had no impact on the %FR in either of the plant species. In *Ranunculus acris* the roots were finer in 2001 than in 2002 ($p < 0.001$), while there was no difference in *Agrostis capillaris* with respect to year (data not shown).

The percentage fraction of fine roots accounted for 9.5% of the variation in the %RLC in *Ranunculus acris* (Linear regression, $p < 0.001$), while there was no significant relationship between %RLC and %FR in *Agrostis capillaris*.

To find a statistically reliable suggestion for which parameters explain most of the %RLC variation, a stepwise regression was carried out. In *Ranunculus acris* %FR and soil nutrients, in particular soil phosphate and nitrogen, were the most important, explaining 25% of the variation, but when H+, Na, K and Ca were added to the

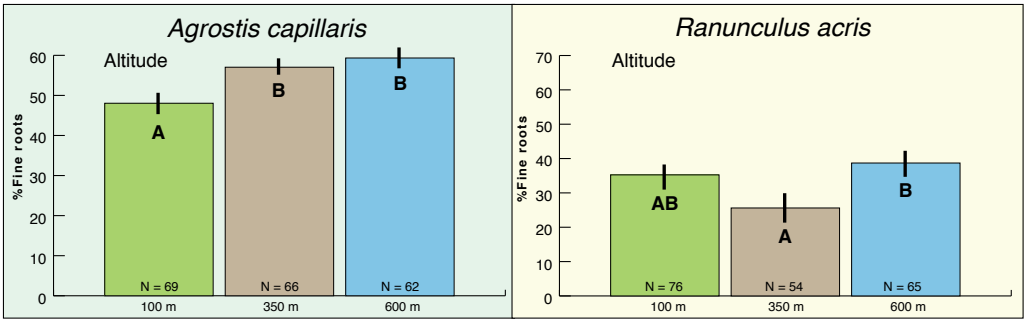

Fig. 3. The percental fraction of fine roots with regard to altitude. Values are means ±SE.

equation, up to 44% of the variation were explained ($p < 0.001$). In *Agrostis capillaris* soil nitrogen and LOI were the most important, explaining up to 15% of the variation ($p < 0.001$). Soil nitrogen and LOI are closely correlated and might therefore both enter the model because of autocorrelation.

The seasonal variations of %RLC differed between the two plant species. *Agrostis capillaris*, flowering in June-July, showed no significant seasonality in colonisation, while *Ranunculus acris*, flowering in May-June, had its lowest ($p < 0.001$) colonisation in June (Fig. 4). The same pattern was found in the percental

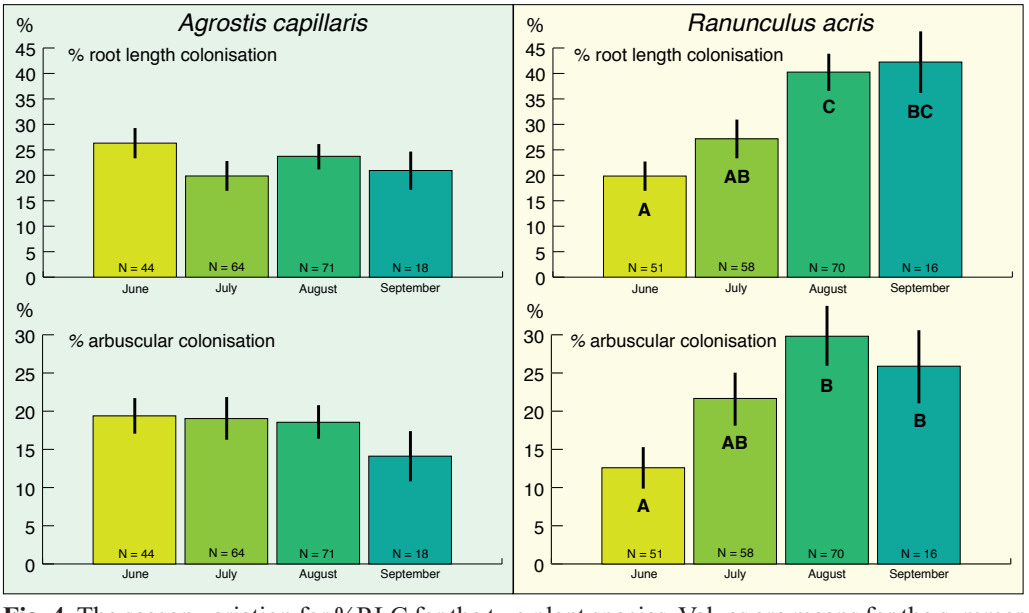

Fig. 4. The season variation for %RLC for the two plant species. Values are means for the summer period 2001 og 2002 (n = 392) ±SE.

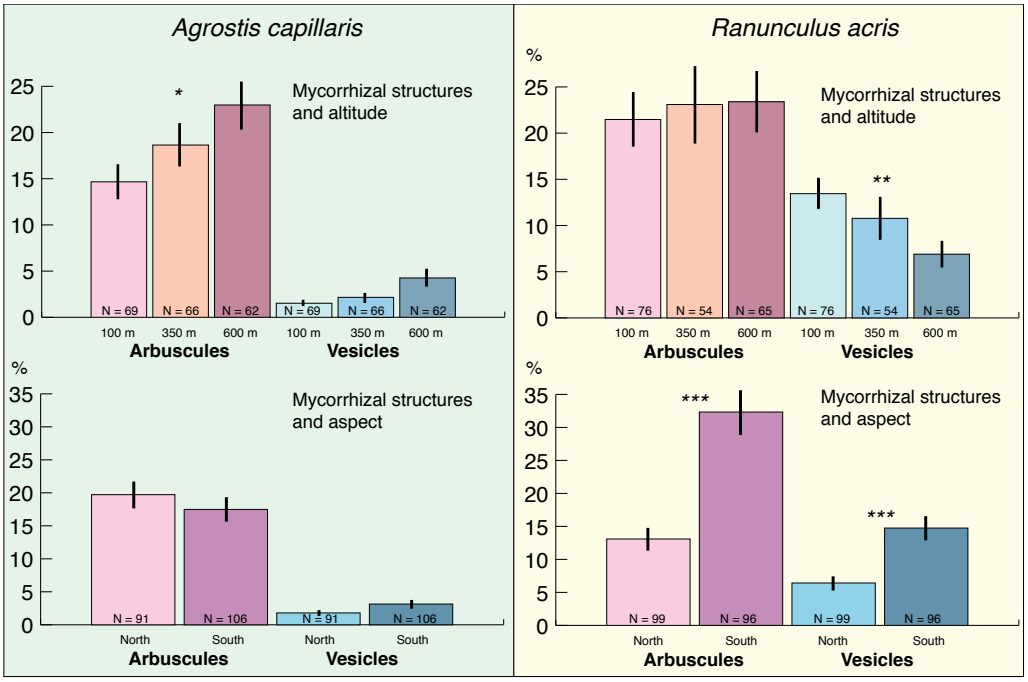

Fig. 5. The mean percental root lengt colonised with vesicles according to plant species and aspect. Values are means (n = 392) ±SE. Red bars indicate the mean of arbuscular colonisation, while blue bars indicate the means of vesicular colonisation.
Stars indicate a difference found in either arbuscular or vesicular colonisation. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

root length with arbuscules observed, with no significant difference in the colonisation in *Agrostis capillaris*, but increasing arbuscular colonisation in *Ranunculus acris* during the summer.

Mycorrhizal structures

The mycorrhizal structures (arbuscules and vesicles) displayed different patterns in relation to plant species and their responses to soil nutrients. The root length colonised by arbuscules showed little variation among plant species (Fig. 5), mean percental root length colonised with arbuscules was 20.7% ±1.15, but vesicles were more abundant in roots of *Ranunculus acris* ($p < 0.001$).

The mycorrhizal structures had different responses to altitude and aspect. In *Agrostis capillaris* the arbuscular colonisation increased with altitude ($p < 0.05$), while no difference was found in *Ranunculus acris* with regard to arbuscular colonisation and altitude. Instead in *Ranunculus acris* the vesicles responded to altitude with fewer vesicles at high altitude ($p < 0.01$). The arbuscular colonisation in *Ranunculus acris* was significantly greater

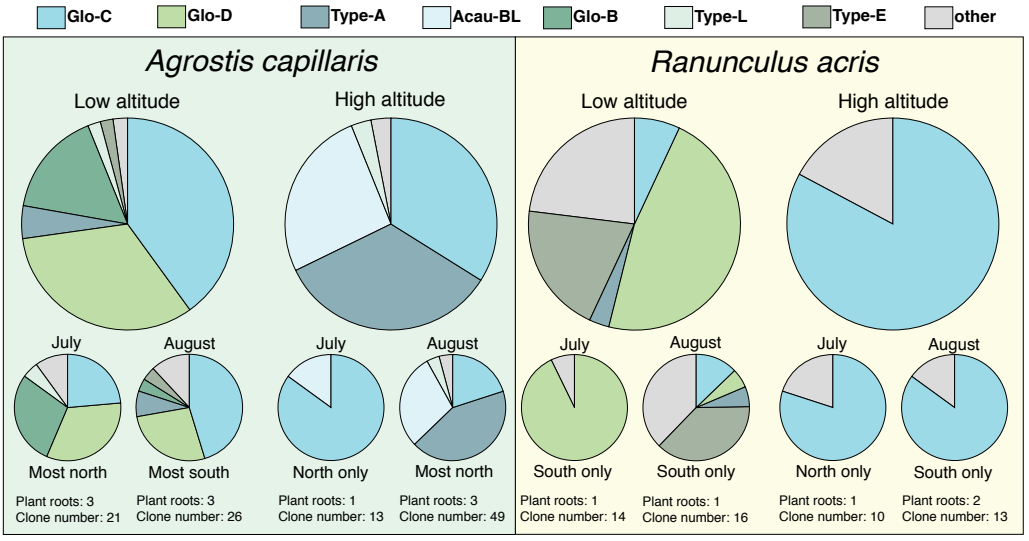

Fig. 6. The sequenced fungal types and their distribution in relation to plant species, altitude, sampling month and aspect. Number of plant roots for each pie is indicated, as is the number of clones. Below the graph is a brief information on the phylogenetic relationship of the sequenced types with known types/species. For further details on the relationship between the different fungal types in this study, see Olsen and Fitter (2004).
Glo-C is identical to the type named Glo3 (UY1227).
Glo-D clusters very distant with *Glomus intraradices.*
Type A clusters distant with *Archaeospora-leptoticha*.
Acau-BL clusters with *Acaulospora capsicula*.
Glo-B clusters together with *Glomus hoi*.
Type-L clusters with *Acaulospora laevis.*
Type E is unidentified, and did not cluster together with any known, culturable fungi.

on southfacing slopes ($p < 0.001$) as were the vesicular colonisation ($p < 0.001$). Soil phosphate concentration was 7.6% greater ($p < 0.001$) on southfacing slopes (Fig. 1).

Molecular data

The clones obtained from these samples represented 7 main types, from which sequences were obtained; a further 11 RFLP patterns were only found once. As the identity of these types is uncertain, they are not included in the analysis performed on the data, but are included in Fig. 6 as 'Other' types.

The most frequent type found in this study is Glo-C, found in both plant species and at both altitudes. Glo-C accounted for 37% of the clones in Fig. 6.

Glo-D was the second most frequent

type, accounting for 18% of the total number of clones; this type is only found at low altitude where it accounted for 39% of the total clones. Type-A was found at both low and high altitude and in both plant species, though most at high altitude and most in *Agrostis capillaris*. Type-E was only found at low altitude, and most in *Ranunculus acris*. Type-B was only found at low altitude, and Acau-BL only at high altitudes. Type-B, Type-L and Acau-BL were only found in *Agrostis capillaris* (Fig. 6).

Of the clones, 70% were obtained from *Agrostis capillaris*.

The hierarchical loglinear analysis approach shown in Table 1-Above demonstrates a very high probability (> 0.9) for a good fit. The partial chi-square values shown in Table 1-Below show the individual combinations. None of the factors used as single factors gave a good fit, nor did any of the two-way interactions. However, two of the three-way interactions had a very good fit. In both cases, it was the interaction Plant*Type in combination with either Aspect or Altitude. Type*Altitude*Aspect was not significant, indicating that the Plant factor is crucial for the model. Thus, loglinear analysis suggests that the pattern of fungal types in the two plant species differ, but that it is in interaction with the habitat.

Discussion

The major finding in the present study was that two plant species differed markedly in mycorrhizal response. While the colonisation in *Agrostis capillaris* responded to nitrogen, *Ranunculus acris* responded to soil phosphate but also to nitrogen. A fine root system as that of *Agrostis capillaris* might, relative to a coarse root system as that of *Ranunculus acris,* indicate that a plant is less dependent upon mycorrhizal colonisation for acquiring phosphate (Fitter and Moyersoen, 1996; Fitter and Merryweather, 1992). Therefore, *Ranunculus acris* appears to be more dependent upon the mycorrhizal symbiont than *Agrostis capillaris* for phosphate uptake, though *Agrostis capillaris* might gain other benefits from the symbiosis, such as protection against pathogens (Newsham *et al.*, 1995a). The present study also suggested that acquiring nitrogen additionally might be linked to the root colonisation.

It is increasingly acknowledged that the AM symbiosis is responsive to both phosphate and nitrogen. If both phosphate and nitrogen are sufficient in the medium, AM root colonisation is suppressed, but no suppression occurs if either of these two nutrients is limited (Blanke *et al.* 2005; Corkidi *et al.* 2002; Sylvia and Neal 1990). For example Chen *et al.* (2014) found that a type called Glo3 decreased on sites where nitrogen fertilizers were applied, in particular if it was together with phosphate.

This Glo3 type is identical to the type named Glo-C in this study, and is found in several previous published work, e.g. Vandenkoornhuyse *et al.* (2002), Husband *et al.* (2002), Helgason *et al.* (1999). This type has been found in tropical as well as in subarctic regions, and most likely has a worldwide distribution.

Colonisation with Glo3 (type C in this study) could indicate that the function of

Backwards eliminations	Factors included	df	Likelihood ratio chi-square	Probability
Step 1	Plant*Altitude*Type Plant*Altitude*Aspect Plant*Type*Aspect Altitude*Type*Aspect	4	0.00007	1.000
Step 2	Plant*Altitude*Aspect Plant*Type*Aspect Altitude*Type*Aspect	8	0.00757	1.000
Step 3	Plant*Altitude*Aspect Altitude*Type*Aspect Plant*Type	12	5.93957	0.919

Factor	df	Partial Chi-sq.	Probability
Plant*Type*Altitude	4	0.008	1.0000
Plant*Type*Aspec	4	0.486	0.9748
Plant*Altitude*Aspect	1	2.577	0.1084
All other combinations had a probability below 0.1.			

Table 1-Above. Hierarchical loglinear analysis showing models of the interactions between the factors 'plant species', 'altitude', 'fungal type' and 'aspect'. Saturated model: Plant * Altitude * Type * Aspect. Type stands for Fungal types and is the factor in question. The best fit of factors interacting for each step is not used in the following step. The data used in this loglinear analysis were those presented in Table 2.10 with aspect as an additional factor. Type L had too few occurrences, and did not enter the analysis. 'Other' types were not included.

Table 1-Below. Partial chi-square values from the loglinear analysis above.

the symbiosis is to acquire in particular nitrogen in nutrient poor soils. *Agrostis capillaris* is flowering in Jun-Jul (Johansen, 2000), while *Ranunculus acris* flowers a month earlier (pers. observation). The difference in flowering time, as well as the fact that *A. capillaris* is a commonly grazed plant (Fosaa and Olsen, 2007), is likely to partly explain the difference in the seasonal pattern of %RLC, since *R. acris* especially in the autumn collects nutrients for the early flowering next spring, while *A. capillaris* needs a steady supply of nutrients during the growth period for flowering and for regrowth due to lost tissue by grazing.

Recent studies have found that arbuscular mycorrhizal fungi contribute to the plant uptake of nitrogen obtained from organic material (Hodge *et al.*, 2001; Leigh *et al.* 2008; Atul-Nayyar *et al.* 2009). This finding might be relevant for this present study, as the Faroese soils are high in both carbon and nitrogen (Fig. 1), and it is likely that the bulk of nitrogen in Faroese soils is organically bound (Olsen and Fosaa, 2002), possibly increasing the importance of the AM symbiosis for retrieving organically bound nutrients.

The mycorrhizal structures arbuscules and vesicles indicated a different colonisation pattern in the two plant species (Fig. 5). Although the %RLC in *Agrostis capillaris* had a significant response to altitude (Fig. 2), only arbuscules had a significant response on their own, though the vesicles showed a similar, but insignificant, pattern, with more vesicles at high altitudes (Fig. 5). On the other hand the vesicles in *Ranunculus acris* displayed a significant response to altitude, which was not found with the %RLC or arbuscules. Hawkes *et al.* (2008) found vesicles to respond to temperature, since they found more vesicles in cooled soils and suggested that this could be the means by which the fungus survives through periods of poor growth conditions. However, they acknowledged that in a multispecies community, individual species of AM fungi are likely to respond differently to temperature, with some more successful than others under colder or warmer conditions. In the present study the vesicles in *Ranunculus acris* were more abundant at warmer sites (e.g. low altitude and southfacing slopes), while the vesicles in *Agrostis capillaris* might have had a similar response as found by Hawkes *et al.* (2008). It is therefore likely that the symbiosis had different strategies in the two plant species, and especially in *Ranunculus acris* the AM fungi might have allocated the photosyntate in vesicles for storage.

The Faroese winters are relative mild, allowing some plants to have a limited growth during winter – this applies for *Agrostis capillaris*, but *Ranunculus acris* has only minimal above-ground structures during winter. Further, *Ranunculus acris* is one of the earliest flowering plants in the Faroes, and it is not unusual that the plants starts their growth before the snow has disappeared. To do so, they need to store carbohydrates in their root tissue during winter, and the high root colonisation in late summer is probably a response to a high nutrient demand during autumn, while the plants prepared for the winter (Fig. 4). This suggestion is in accordance with findings by Olsson et al. (2011) demonstrating that vesicles contain in particular phosphorus in much greater concentrations than any other element.

The method of distinguishing between fine and coarse roots with two different critera (diameter and development of the stele) might seem a bit confusing, especially since there is no consensus on how to classify and measure fine roots (Tobner *et al.*, 2013). However, the approach used in this study is quite similar to the functional classification approach suggested in McCormack *et al.* (2015) where the absorptive capacity is the function of interest, and this occur mainly in the

roots of 1st order, or the finest roots.

In general, *Ranunculus acris* had coarser roots than the grass (Fig. 3), as expected, and it was in particular the finer roots that were colonised. In *Agrostis capillaris* the roots were coarser at low altitude, where the soil nitrogen content was greatest. The finer roots at higher altitudes could be because the roots proliferated more in their search for nutrient rich patches as explained by Hodge (2006).

The number of AM fungal types found in roots from *Agrostis capillaris* was greater than in roots from *Ranunuculus acris* (Fig. 6). However, this is probably explained by the poor amplification success rate, as twice the number of roots from *Agrostis capillaris* amplified relative to samples from *Ranunculus acris*. Even so, the AMF types showed some patterns in relation to altitude, with more types at low altitude in both plant species, and some AMF types more abundant in either of the plant species.

The only AMF type that was found to be identical to types found in other studies was Glo-C (Glo3). This was the most common AMF type, found at both low and high altitude, and in both plant species regardless of aspect or sampling date. This particular AM fungi has been brought into culture, and in a laboratory study it was found only to contribute with a small increase in phosphate uptake, if any, and not colonise all plant species tried (Helgason *et al.*, 2002). The statistical analyses suggested that nitrogen was an important factor for the root colonisation in both plant species, and Glo-C might be better in facilating nitrogen uptake than phosphate, and should be studied further.

Another AMF type in this study is Glo-B, who is quite similar to *Glomus hoi*, which has been found to be quite efficient in improving phosphate uptake and enhancing plant growth (Helgason *et al.*, 2002). *Glomus hoi* has also been found to be able to capture and transfer nitrogen from organic patches at 10-12°C (Barrett *et al.*, 2011), which is comparable to the Faroese environmental summer temperature.

The data support the hypothesis that plants from a single habitat that differ in mycorrhizal dependency would be colonised by distinct fungal communities. The data shown in Table 5 indicate that some fungal types might have preferences towards one plant species, while other fungal types might be sensitive to altitude. This is supported by the model applying log-linear analysis: Removing the plant species from the model generated a poorer fit, though a good fit also required a factor representing the habitat. This finding is another indication that the earlier view that any AM fungus would colonise any plant needs to be reconsidered, and that the fungal community probably is a reflection of plant community, nutrient availability, temperature, as well as other biotic and abiotic factors.

Acknowledgements

The author thanks Alastair H. Fitter for valuable comments on the manuscript, and the staff at the University of York for help with the molecular analyses. This work was funded by The Faroese Research Council.

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