Habitat preferences of the arbuscular mycorrhizal fungi of the Faroes compared with other places

Val av lívøki hjá arbuskulsoppum í Føroyum sammett við aðrastaðni

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Úrtak

Arbuskul soppar eru megin-mycorrhiza symbiosan í føroysku vegetatiónini. Ymiskir soppar eru funnir í ymiskum vertsplantum, og harafturat er munur á hvør soppur er í sama plantuslagi frá ymsum støðum. Her verða framløgd úrslitini av tí, sum er funnið í vanligum fínagrasi (*Agrostis capillaris*) sammett við tað, sum onnur hava funnið í somu plantu aðrastaðni. Roknað varð við at føroyska arbuskul soppa floran líktist tí sum er funnið í Skotlandi har eisini vanligt fínagras var kannað, men so er ikki. Hinvegin er munurin á tveimum føroyskum støðum líka stórur sum munurin á føroyskum støðum og kannaðum stað í Skotlandi. Føroyska arbuskul soppafloran líkist mest tí sum er funnið í einum enskum skógi.

Abstract

Arbuscular mycorrhiza (AM) is the main symbiosis in the Faroese vegetation. The fungal types found in different host plants differ, and there is also a difference of fungal types in the same plant species from place to place. Here the results from a survey of fungal types in *Agrostis capillaris* are compared with what other studies have found. It was expected to find a similar AM fungal flora as in a study from a grassland in Scotland, also using *Agrostis capillaris* as the host plant, but that was not the case. On the other hand two Faroese sites differ as much as the Faroes and the Scottish site. The Faroese AM fungal flora sampled here resembled most closely that found in an English forest.

Introduction

It is the rule rather than the execption for a plant so be mycorrhizal. If we take the Faroes as an example, then approx. 70% of all the plant species are AM, while much of the rest is ecto- or ericoid. Several plants are non-mycorrhizal but when considering their frequency they only account for 6-7% (Olsen and Fosaa, 2002). These numbers fits reasonably well with what others have found in other places. It is widely accepted that around 2/3 of all plant species worldwide are arbuscular mycorrhizal (AM) (Fitter and Moyersoen, 1996).

One benefit of being mycorrhizal for the plant is increased nutrient uptake, especially phosphorus (Fitter, 1985), but there are also other gains of the symbiosis, e.g. pro-

tection against pathogens (Newsham *et al.*, 1995a). For the fungi the benefits are obvious. The AM fungi are unable to obtain carbohydrates from other sources than from the plant root, because they are obligate symbionts. They have an internal and an external phase. The internal phase consists of hyphae, arbuscules and vesicles. Arbuscules are "tree-like" structures and consist of highly branched very fine hyphae. Vesicles are storage organs, and are swollen either hyphal ends or attached laterally to hyphae. The external phase consist of hyphae and spores. The external hyphae can grow several centimetres from the plant root, allowing transport of phosphate from outside the zone of depleted soil that surrounds the root, and this is considered as the main benefit for the plant. Terrestrial systems are often limited by phosphorus, which is one of the most immobile nutrients in soil. Therefore, under phosphorus limited conditions, it is very valuable for the plant to be connected to some fungi that with their fine hyphae are able to explore the surrounding soil for nutrients much better than the plants relative coarse roots can.

AM fungi disperse with external hyphal growth. However they also produce spores, and while the external hyphae can be considered as dispersal through space, then the spores can be considered as dispersal through time (Fitter and Garbaye, 1994).

One of the confusing things about AM fungi is the taxonomy. By definition the taxonomy is done from spores which can be found in the soil. However, several studies have shown, that the spores extracted from the soil do not reflect the fungi found colonising the roots (Clapp *et al.*, 1995; Merryweather and Fitter, 1998). Some fungi produce many spores, other few or none. Therefore in ecological studies the question of diversity might be of more importance than the taxonomy.

The accepted method of identifying these fungi is to describe their spores, and hitherto are less than 200 AM fungal species in total described (Morton and Benny, 1990). This is probably the main reason behind the belief that the AM fungi are generalists, and able to colonise nearly whatever plant species is present. However, more and more evidence is revealed indicating that this is not the case. For example AM fungi, if not specific in their host choice, are at the least selective (Fitter *et al.*, 2004). Further, a profound difference has been found in the occurence of the AM fungal species at low and high altitude in the Faroes (this study).

If only 200 AM fungal species are able to colonise around 200,000 plant species we would indeed have generalistic fungi. If it is so we should expect the same fungi to be found worldwide, with only slightly consideration to habitat. The preliminary findings from my molecular work is that the fungal types found in a root varies with both host plant, altitude and aspect; or host and habitat, but which or these is the main factor determining the arbuscular fungal flora?

Material and Methods

Material

The plant species used in this study is

Agrostis capillaris, in most case growing with *Ranunculus acris*. The fungi therefore had the opportunity to colonise both plant species. The same main AM fungal types are found in both plant species, but they show different patterns according to habitat, and some AM types show preference towards a specific host (unpublished data). Sequences from these field-collected fungal types were compared with sequences submitted to the Genebank of the National Center for Biotechnology Information (NCBI) from a similar study in Scotland (Vandenkoornhuyse *et al.*, 2002; Genbank accession nos AF437637-AF437723). The Vandenkoornhuyse study used very similar methods as the study presented here for the molecular work, and their host plant was also *Agrostis capillaris,* though growing with *Trifolium repens*.

For identification purposes named sequences are used in the phylogenetic tree (Fig. 1). These were submitted by Schüßler *et al.* (2001) and Helgason *et al.* (1999; 2002), and obtained from NCBI.

The results from plants of *Agrostis capillaris* from 2 different sites in the Faroes were compared with *Agrostis capillaris* from a Scottish site when all were grown in a glasshouse.

The sites

The Faroese sites consist of 4 altitudinal slopes, 2 north-facing and 2 south-facing. The results provided here are taken from the lowest altitude, approx. 100 m asl, and the highest 600 m, in total 8 sites. The sites are open grassland and span from temperate zone (south-facing low altitude, to alpine zone (all high altitude sites) (Fosaa, 2004). The sites are all grazed by sheep.

The glasshouse grown *A. capillaris* are from 1 north-facing high altitude site (Sornfelli), 1 south-facing low altitude site (Velbastað) and 1 Scottish low altitude site (Sourhope).

The "Sornfelli" site is a north-facing high altitude site (600 m a.s.l.), "Velbastað" is low altitude (200 m a.s.l.) south-facing, while "Sourhope" is low altitude (300 m a.s.l.). The Sourhope sampling was on the top of a hill. Average soil temperatures at Sourhope range from 7.7-8.7°C (Burt-Smith, 1999-2002), while a 3 year mean of the soil temperature at Sornfelli was 4.2°C (Fosaa *et al.*, 2002). No temperature logging is from Velbastað, but south-facing sites at the same altitude range from 6-6.6°C (Fosaa *et al.*, 2002). All temperature measurements are annual mean temperatures. All the sites are grazed by herbivores, the Faroese sites mainly by sheep, the Scottish site by cattle.

The data from glasshouse grown plants are based upon 4 successful amplifications from Sornfelli, 3 from Velbastað and 8 from Sourhope. The sampling dates differ, the Faroese samples were taken in late August, while the Scottish samples were taken late October. The difference in sampling date should be overcome by the treatment the sampled plants and fungi have had: they were grown for several months (6 months for the Faroese samples, 4 months for the Scottish) under the same conditions: 10-12°C in a chilled counter located in a warmed glasshouse with light on 16 hours.

Field work

During the summer 2002, 3 cores of soil, approx 5-6 cm wide and 8 cm deep with *Agrostis capillaris* were taken from each site at each sampling day. The roots were washed, dried and stored at room temperature until extraction.

The data are based upon two sampling dates for each site. Three plant roots were sampled and the DNA is extracted from a pool of the three roots. From one of the low altitude south-facing sites no PCR product was obtained from one sampling date, while from the other low altitude south-facing site the cloning was not very successful, providing only 3 clones. Therefore the north-facing sites might be overrepresented (Table 1).

For the lab grown *A. capillaris* 8 samples from Sourhope and 8 from Sornfelli were taken, but only 4 from Velbastað. The success rate for the DNA amplifications was 50% for Sornfelli, 75% for Velbastað and 100% for Sourhope.

DNA extraction

The DNA was extracted by the CTAB method (Edwards *et al.*, 1997). Roots were ground in liquid nitrogen, and then the DNA was extracted by phenol and chloroform. After washing with ethanol the extract went through an extra purification with StrataPrep PCR Purification Kit from Stratagene. These purifications are necessary to get rid of inhibitors.

The DNA from the samples used in the T-RLFP analysis was extracted with the Qiagen DNeasy Plant Mini Kit according to the manufacturer's protocol.

PCR

The genes here amplified with PCR are those that define the ribosomal RNA (rRNA). For this purpose TAQ polymerase (Invitrogen) was used together with the primers NS31 (Simon *et al.*, 1992), AM1 (Helgason *et al.*, 1998) and AM2 (Ridgway, unpubl.). NS31 is an general primer, valid for all eucaryotes, while AM1 is a specific primer, amplifying especially glomalean fungal types, but not paraglomalean and *Archaeospora* types. AM2 amplifies a shorter segment and a wider range of glomalean fungi, paraglomalean and *Archaeospora* types. The drawback of using AM2 is that more ascomycetes are amplified together with the glomalean fungi.

The reactions were performed using 0.2 mM dNTPs, 10pmol of each primer and the supplied reaction buffer to a final volume of 50 µL. The PCR-amplification was done on a PTC-200 machine (MJ Research), 94°C for 3 min, then 30 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min. The final step was 10 min. at 72°C.

The T-RLFP samples were amplified using Qiagen Multiplex PCR Kit, 5 pmol of each primer (AM1 and NS31). Q-solution, a reagent modyfying the melting behaviour of DNA (Qiagen), was added according to the manufacturer's protocol. Amplification on a PTC-200 machine, 95°C for 15 min., 40 cycles with 94°C for 30 sec., 58°C for 90 sec. and 72°C for 90 sec. The final extension was 72°C for 10 min.

Cloning

The PCR product was inserted into a pGEM-T vector (Promega), and then the

recombinant DNA molecule was transferred into competent *E. coli* cells (DH5α from Gibco BRL Life Technologies). All steps strictly followed the manufacturers' manual. The competent cells were grown on solid medium, forming colonies of identical clones. At least 16 clones were used for each sample, giving a total of 244 clones.

RLFP

The clones were grouped by using the enzymes *Hin*fI and *Hsp*92II (Promega) mainly to distinguish between the glomalian types, while *Stu*I and *Hpa*II were used to detect amplified ascomycetes. *Hpa*II did also distinguish between some acaulospora types. When all the ascomycetes were omitted, only 127 clones were left.

T-RLFP

The results where an English site (Sourhope) is compared with Faroese sites are obtained by T-RLFP (Liu *et al.*, 1997). The PCR products were digested with *Hin*fI and then run on a Beckman Coulter CEQ8000. The fragment length was analysed with Beckman Coulter CEQ8000 software (from Beckman Coulter).

Sequencing

Representatives for each main glomalean group were sent for sequencing at the Technology Facility, University of York, UK. Only one strand was sequenced. The sequencing was done on a Beckman Coulter CEQ8000 analyser.

Statistics

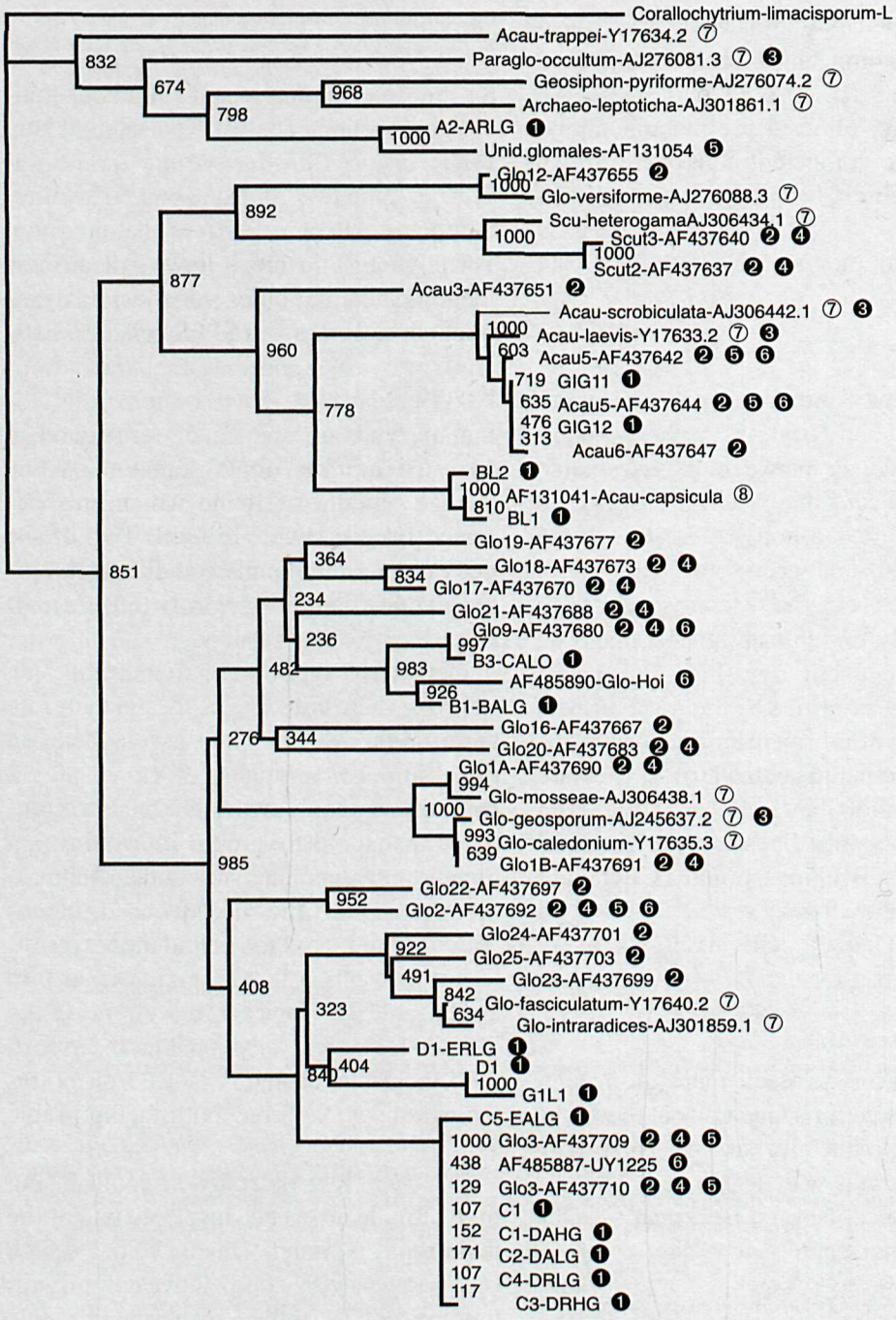
ClustalX (Thompson *et al.*, 1997) was used for multiple alignment and neighbour-joining phylogenetic analysis (Saitou and Nei, 1987), using *Corallochytrium limacisporum*, a putative choanozoan (Cavalier-Smith and Allsopp, 1996) as the outgroup. The phylogenetic tree is drawn in TreeView (Page, 1996). All other statistical analyses were carried out using SPSS version 11.01.

Results

5 main types of AM fungi were found in *Agrostis capillaris* roots (Table 1). A further 10 types were found which only occurred once or twice in total. Two of the main types were mainly found at high altitude, and two other were only found at low altitude.

In total 16 types were found, although not all of them are sequenced, and therefore there might still be some ascomycetes in the group. The unsequenced clones are 12 in total, so their importance is low compared with the main group. To avoid a high Shannon index due to undetected ascomycetes, all the unsequenced clones were omitted from the calculations, resulting in a Shannon index ranging from 0 to 1.19 (Table 1). However, if we remove the altitude factor and only consider the aspect, then the Shannon index is 1.63 on north-facing slopes, 0.45 on south-facing (Table 2).

The very low diversity at south-facing high altitude arises because only one of the main types is found. This is "Glo3" which seems to be a very competetive colonisator, present in all studies mentioned in this


Corallochytrium-limacisporum-L
Acau-trappei-Y17634.2 ⑦
Paraglo-occultum-AJ276081.3 ⑦ ❸
Geosiphon-pyriforme-AJ276074.2 ⑦
Archaeo-leptoticha-AJ301861.1 ⑦
A2-ARLG ❶
Unid.glomales-AF131054 ❺
Glo12-AF437655 ❷
Glo-versiforme-AJ276088.3 ⑦
Scu-heterogamaAJ306434.1 ⑦
Scut3-AF437640 ❷ ❹
Scut2-AF437637 ❷ ❹
Acau3-AF437651 ❷
Acau-scrobiculata-AJ306442.1 ⑦ ❸
Acau-laevis-Y17633.2 ⑦ ❸
Acau5-AF437642 ❷ ❺ ❻
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Acau5-AF437644 ❷ ❺ ❻
GIG12 ❶
Acau6-AF437647 ❷
BL2 ❶
AF131041-Acau-capsicula ⑧
BL1 ❶
Glo19-AF437677 ❷
Glo18-AF437673 ❷ ❹
Glo17-AF437670 ❷ ❹
Glo21-AF437688 ❷ ❹
Glo9-AF437680 ❷ ❹ ❻
B3-CALO ❶
AF485890-Glo-Hoi ❻
B1-BALG ❶
Glo16-AF437667 ❷
Glo20-AF437683 ❷ ❹
Glo1A-AF437690 ❷ ❹
Glo-mosseae-AJ306438.1 ⑦
Glo-geosporum-AJ245637.2 ⑦ ❸
Glo-caledonium-Y17635.3 ⑦
Glo1B-AF437691 ❷ ❹
Glo22-AF437697 ❷
Glo2-AF437692 ❷ ❹ ❺ ❻
Glo24-AF437701 ❷
Glo25-AF437703 ❷
Glo23-AF437699 ❷
Glo-fasciculatum-Y17640.2 ⑦
Glo-intraradices-AJ301859.1 ⑦
D1-ERLG ❶
D1 ❶
G1L1 ❶
C5-EALG ❶
Glo3-AF437709 ❷ ❹ ❺
AF485887-UY1225 ❻
Glo3-AF437710 ❷ ❹ ❺
C1 ❶
C1-DAHG ❶
C2-DALG ❶
C4-DRLG ❶
C3-DRHG ❶
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Fig. 1

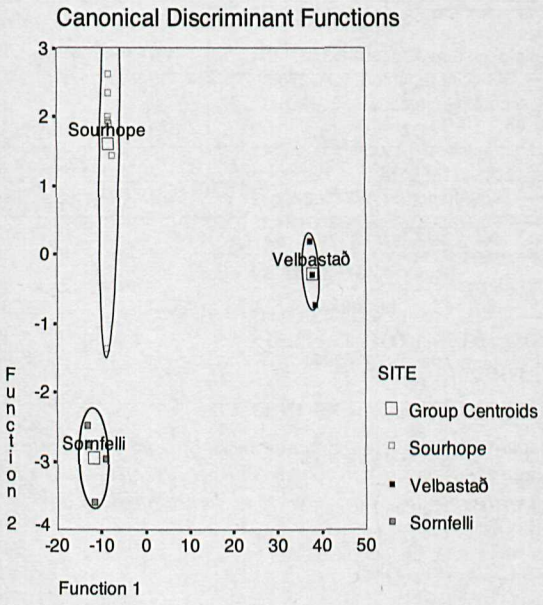

Fig. 2. Canonical Discriminant analysis of the T-RLFP values from 2 Faroese sites and one Scotish, all grazed grassland (Sornfelli: north-facing, high altitude; Velbastað: south-facing, low altitude; Sourhope: scottish, low altitude). The three sites have clearly distinct AM communities.

study and also in most of the host plants.

The phylogenetic tree (Fig. 1) is based on the region amplified by the AM2 primer; meaning that the basepairs between AM2 and AM1 are omitted. However, since most of the variation lies between 80-300 bp from the 5' (NS31) of the PCR product, most of the variation should be retained (Helgason *et al.*, 1999).

The T-RFLP results (Fig. 2) indicate that the fungal communities in the sites are distinct. In contrast to the clone-work only *Hin*fI was used, and only the strand from NS31 considered. This is due to the fact that a very large fraction of the AM fungi have a *Hin*fI site directly after the AM1 primer, and therefore no results are obtained from the AM1-labelled end. Previous attempts also showed that *Hsp*92II didn't give any additional information, since most of the types were cut into the same pattern.

Discussion

Results from clones

Only one previous molecular study of AM fungi appears to have used *Agrostis capillaris* as the target plant: Vandenkoornhuyse (2002) examined *A. capillaris* and *Trifoli-*

Fig. 1. *Phylogenetic (Neighbour-joining) tree showing all the glomalian fungi found in Agrostis capillaris. The numbers in circles indicate where else these types have been found.*
1: Sequences obtained from Faroes
2: Sequences obtained from Sourhope (Genbank: accession nos: AF437637-437723, Vandenkooornhuyse et al., 2002)
3: Spores from Shetland identified as the named type (Watling, 1992)
4: Sequences obtained from a Tropical Forest (Genbank: accession nos: AY129570-129633; Husband et al., 2002)
5: Sequences obtained from a British Forest (Genbank accession nos: AF131020-131055; Helgason et al., 1999)
6: Sequences obtained from a British Forest (Genbank accession nos: AF485861-485890; Helgason et al., 2002)
7: Sequences obtained from identified spores (Genbank accession nos on the graph; Schüßler et al., 2001)
8: Sequence obtained from library culture (see Helgason et al., 1999)

Abbreviations: Glo: Glomus; Acau: Acaulospora; Scu: Scutellospora; Paraglo: Paraglomus; Archaeo: Archaeospora.

Aspect	Altitude	#A	#B	#C	#D	#BL	#Other	**Sum**	[P] in soil (mg/kg)	Temp. (°C)	S-W	# Clones	# AM types
North	Low	0	6	11	14	0	3	**34**	10.3	6	0.95	27	4
North	High	21	0	11	0	16	4	**52**	13.8	3.5	1.19	50	4
South	Low	2	1	7	0	0	2	**12**	26.0	6.8	0.80	10	3
South	High	0	0	26	0	0	3	**29**	17.5	4	0	26	1
		23	**7**	**55**	**14**	**16**	**12**	**127**					

Chi-square calculations for all categories of AM fungal types: df = 15, chi-square: 123.478, $p < 0.001$

If only aspect is considered, then df = 5, chi-square = 54.052 and $p < 0.001$

If only altitude is considered, then df = 5, chi-square = 41.004 and $p < 0.001$

Table 1. *Number of the main AM fungal types and a Chi-square test, indicating that al the AM fungal communities vary according to both aspect and altitude. Further are listed the values of extractable phosphorus (method: Olsen et al, 1954 and Banderis et al., 1976), means of onsite temperature loggings, number of clones for each aspect/ altitude category and the number of AM fungal types.*

um repens from a seminatural grassland at the Macaulay Institute's Sourhope field station in Scotland, and concluded that the two plant species host different AM fungi, but that *A. capillaris* supported 21 different AM types.

The Shannon-Wiener index (Table 1) shows a very low diversity at high altitude south-facing. The explanation might be that at high altitude there is reasonable more extractable phosphate on the south-facing sites than at the north-facing. There is also a temperature difference which migth affect the growth of both plant and fungi (Table 1). The Shannon index was 1.71 for the Sourhope grassland ecosystem (2 plant species).

	S-W	# Clones	# AM types
High	1.30	76	4
Low	1.07	36	5
North	1.63	82	6
South	0.45	37	4

Table 2. *Shannon-Wiener values when only altitude is considered (row 1 and 2) and when only aspect is considered (row 3 and 4).*

Studies comparing plants with fine versus coarse root systems show that plants with fine root systems might be less dependent upon the mycorrhizal symbiont for phosphate uptake than plants with coarse roots (Newsham *et al.*, 1995b). As most grasses, *Agrostis capillaris* have a fine root system, and might be able to control the colonisation under "high" phosphate levels.

There is higher diversity at the north-facing sites, and at high altitude compared with low altitude. These sites have in common lower temperature and lower phosphate content than low altitude and south-facing sites. AM fungi are known to increase the host plants phosphate uptake,

and therefore a higher colonisation rate is frequently observed in low phosphate soils compared with nutrient rich soils. This trend might also be observed with the types of AM fungi colonising the roots, for example Helgason *et al.* (1998) found that the Shannon index was 1.44 for old woodland soil and 0.39 for arable soil.

The sampling intensity of the Vandenkoornhuyse's study was considerable higher than in the present study, and they claim that if the sampling is insufficient then that will lead to decrease in the number of phylotypes found (Vandenkoornhuyse, 2002). It is therefore likely that the sampling from the Faroes would have revealed more types if the number of clones was increased.

The Faroese AM fungi from *Agrostis capillaris* do not group particularly well with fungi found in *Agrostis capillaris* at Sourhope. There are three types in common, two of which are *Glomus* spp., Glo3 and Glo9, but these are found in all four studies compared in Fig. 1 and Table 3, indicating a worldwide distribution. Further, one Acaulospora (Acau5), which also is found in the "English forest" studies, occurs in the Faroes. Table 3 and Fig. 3 are based on the data used to compare the Faroese data with in Fig. 1. Fig. 3 indicates that the Faroese AM fungal flora is most similar to what is found in an English forest. The Scottish *Agrostis capillaris* AM fungal flora seems to be as distant as the tropical forest to Faroese and English sites.

	Sourhope	**Faroes**	**Panama**	**England**
Number host plants	2	2	2	6
Number clones	2001 (1696)	176 (127)	1383	257
pH range	4.3-5	4.8-6	n.a.	2.9-3.4*)
Glomaceae	19 (16)	5 (5)	22	7
Acaulosporaceae	3 (3)	2 (2)	4	5
Gigasporaceae	2 (2)	0	4	1
Archaeosporaceae/Paraglomaceae	0	1 (1)	0	1
Total number of types	24	8	30	14

Table 3. *Number of AM fungal types separated into the different AM fungal taxa. The numbers in brancets are the numbers for Agrostis capillaris only. Only 8 types are sequenced from the Faroes, but in addition to the sequenced types, 10 unidentified RLFP types are found. *) From Farley and Fitter, 1999.*
Sourhope: Scottish grassland with Agrostis capillaris (1 site)
Faroes: Faroese grasslands with Agrostis capillaris (8 sites)
Panama: Tropical forest (2 sites)
England: English forest (3 canopies)

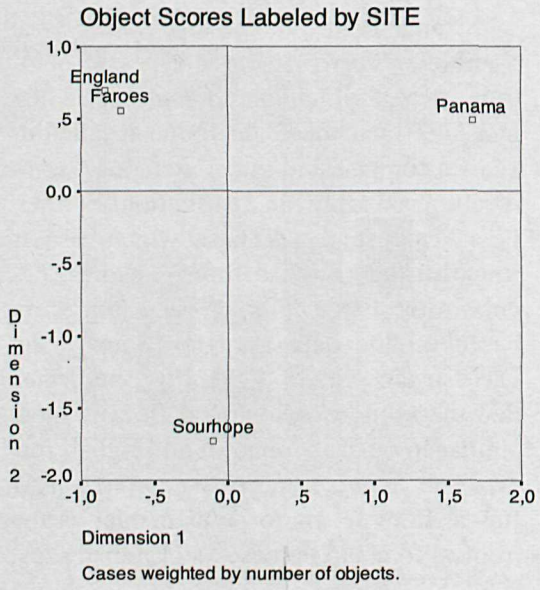

Fig. 3. *Homogeneity analysis of the data summarised in Table 3. The calculations are done from present/absent values.* Sourhope: *Scottish grassland with Agrostis capillaris (1 site).* Faroes: *Faroese grasslands with* Agrostis capillaris *(8 sites).* Panama: *Tropical forest (2 sites).* England: *English forest (3 canopies)*

Results from T-RFLP

A more direct (and efficient) method to estimate diversity is to use the T-RLFP technique. This method allows assessment of diversity without identifying the single types by cloning. One peak can be assumed to be one type – although there might be overlaps with restriction sites at the same place.

Vandenkoornhuyse *et al.* (2003) used T-RFLP analysis to estimate AM fungal diversity in *Agrostis capillaris,* which was 7.54 fungal types per sample. They used 2 PCR amplifications of each sample and 2 enzymes, *Hin*fI and *Hsp*92II and ran each digest twice on the analyser. This method allowed them to distinguish between very small peaks and background noise. The present study was not as thorough, so the data are probably more reliable if cutting off peak height – for example only accepting peaks larger than 3% of the total fluorescence. However, the number of types Vandenkoornhuyse *et al.* found fits well with what this study would get if most of the peaks were valid (Table 3, cut-off peak height 0%). The table indicates that the diversity in Faroese sites is high; even the most restricted criterion (cut-off peak height 5%) indicates a higher number of AM fungal types at low altitude than at the the Scottish low altitude site. This result is in reasonable accordance to the finding from the clonal work, that there are more types at low altitude compared with high (Table 2), especially when considering the limited number of clones from low altitude.

	Peak height			
Cut-off peak height	>0%	>1%	>3%	>5%
Sornfelli	13.75	6.75	3.75	3.25
Velbastað	10	10	8,25	4,75
Sourhope	7.875	5.75	4.625	3.875

Table 4. *Mean number of peaks above a minimal-cut-off peak height. Each peak indicates a possible AM fungal type.*

Conclusion and further discussion

The question raised in this paper is whether the main factor determining the nature of the arbuscular fungal flora is the plant host

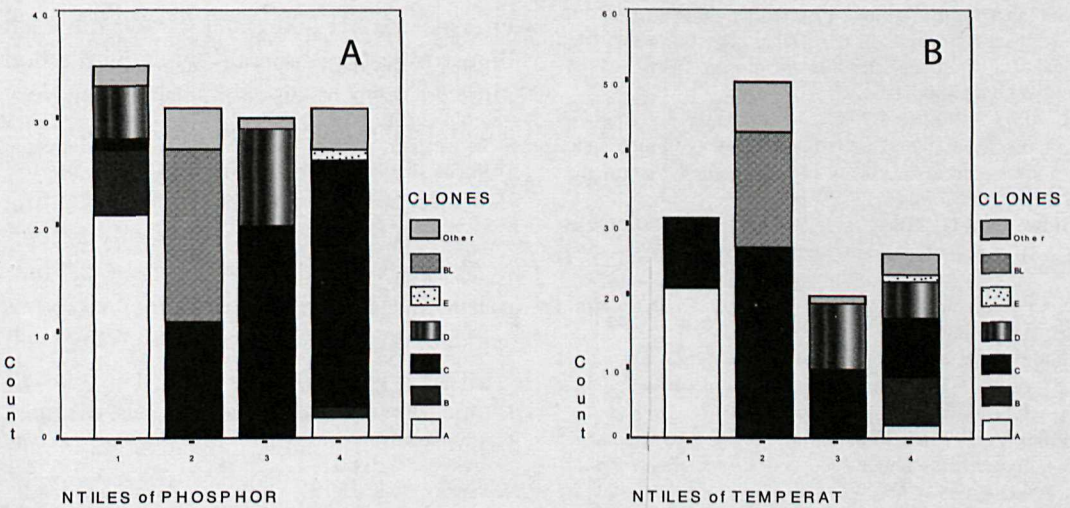

Fig. 4. Number of AM fungal clones from field grown plants. Each type is grouped into 4 categories. The categories are done automatically in SPSS and indicate levels of a) extractable soil phosphorus, b) soil temperature. 1 is the category with the lowest values, 4 has the highest values., either 4 increasing levels of extractable phosphorus (a) or 4 increasing levels of the mean annual temperature (b).
*Anova tests: phosphorus * fungal types F = 7,530, df = 3, p < 0.001; temperature * fungal types F = 9.421, df = 3, p < 0.001; (phosphorus * temperature) * fungal types F = 16.477, df = 6, p < 0.001.*

or the habitat. The data presented here do not indicate any clear host-specific relationship. Both the samples taken directly from the field and the lab-grown samples indicate that there is a larger difference between the sites with *Agrostis capillaris* than between the Faroese samples and samples from English forests.

Other factors which might influence fungal distribution are for example available phosphate and temperature, and indeed some of the AM types seems to relate to the amount of extractable phosphate, for example type C (Glo3) increases with increasing phosphate (Fig. 4). Other might respond to temperature, for example type D and B (Glo9), which are only found at the warmer plots, while A and BL mainly are found at colder plots.

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