

1   **Arbuscular mycorrhizas are present on Spitsbergen**

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3   **K.K. Newsham (✉) • P.B. Eidesen • M.L. Davey • J. Axelsen • E. Courtecuisse • C. Flintrop • A.G.**  
4   **Johansson • M. Kiepert • S.E. Larsen • K.E. Lorberau • M. Maurset • J. McQuilkin • M. Misiak • A. Pop •**  
5   **S. Thompson • D.J. Read**

6   *Department of Arctic Biology, the University Centre in Svalbard, P.O. Box 156, N-9171 Longyearbyen, Svalbard*

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8   K.K. Newsham

9   *British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge,*  
10   *CB3 0ET, United Kingdom (e-mail: kne@bas.ac.uk, tel.: +44 (0)1223 221400)*

11

12   D.J. Read

13   *Department of Animal and Plant Sciences, The University of Sheffield, Western Bank, Sheffield, S10 2TN, United*  
14   *Kingdom.*

15

16   **Abstract** A previous study of 76 plant species on Spitsbergen in the High Arctic concluded that structures  
17   resembling arbuscular mycorrhizas were absent from roots. Here, we report a survey examining the roots of 13  
18   grass and forb species collected from 12 sites on the island for arbuscular mycorrhizal (AM) colonisation. Of the  
19   102 individuals collected, we recorded AM endophytes in the roots of 41 plants of 11 species (*Alopecurus*  
20   *ovatus*, *Deschampsia alpina*, *Festuca rubra* ssp. *richardsonii*, putative viviparous hybrids of *Poa arctica* and *P.*  
21   *pratensis*, *Poa arctica* ssp. *arctica*, *Trisetum spicatum*, *Coptidium spitsbergense*, *Ranunculus nivalis*, *R.*  
22   *pygmaeus*, *R. sulphureus* and *Taraxacum arcticum*) sampled from 10 sites. Both coarse AM endophyte, with  
23   hyphae of 5–10 µm width, vesicles and occasional arbuscules, and fine endophyte, consisting of hyphae of 1–3  
24   µm width and sparse arbuscules, were recorded in roots. Coarse AM hyphae, vesicles, arbuscules and fine  
25   endophyte hyphae occupied 1.0–30.7%, 0.8–18.3%, 0.7–11.9% and 0.7–12.8% of the root lengths of colonised  
26   plants, respectively. Principal component analysis indicated no associations between the abundances of AM  
27   structures in roots and edaphic factors. We conclude that the AM symbiosis is present in grass and forb roots on  
28   Spitsbergen.

29

30   **Keywords** Arbuscular mycorrhizas • High Arctic • Spitsbergen • Svalbard

31

32      **Introduction**

33      The arbuscular mycorrhizal (AM) symbiosis, formed between roots and fungi in the Glomeromycotina and  
34      Mucoromycotina (Spatafora et al. 2016; Orchard et al. 2017), is central to the survival and reproduction of the  
35      majority of terrestrial plant species (Smith and Read 2008). The symbiosis enhances plant uptake of limiting  
36      nutrients, chiefly phosphorus (P), from soil (Smith and Read 2008), with the uptake of P and other elements  
37      being facilitated by the formation in root cortical cells of branched arbuscules. Intracellular and extracellular  
38      vesicles are also formed for the storage of fats, leading to the development of intraradical structures that are  
39      readily identifiable by microscopy.

40      The AM symbiosis is widespread in grass and forb roots in temperate grasslands, dry scrub and deserts,  
41      tropical savannahs and Mediterranean vegetation, and is also found in the roots of trees in tropical rainforests  
42      and tropical seasonal forests (Read 1991). Its abundance declines markedly in cold regions, probably owing to a  
43      reduction in suitable host plant taxa, or the effects on the fungal symbionts of abiotic factors associated with  
44      increasing latitude, such as lower temperature, water availability or growing season length (Upson et al. 2008;  
45      Newsham et al. 2009). The symbiosis has nevertheless been recorded in Arctic habitats. In the Canadian High  
46      Arctic, where mean annual air temperatures range from -18 °C to -14 °C and mean summer air temperatures  
47      from -2 °C to 1 °C (Olsson et al. 2004), abundant AM endophytes (21–94% root lengths colonised) have been  
48      recorded in the roots of six of seven Asteraceae species in the Geodetic Hills on Axel Heiberg Island at 80 °N  
49      (Allen et al. 2006). Frequent AM colonisation (8–85% root lengths colonised) has been found in the roots of  
50      *Erigeron* and *Taraxacum* species from Ellesmere Island at 82 °N (Ormsby et al. 2007), and AM endophytes have  
51      also been found to be commonplace (37–85% root lengths colonised) in the roots of *Arnica*, *Erigeron* and  
52      *Potentilla* species sampled from Banks Island at 73 °N (Olsson et al. 2004).

53      In a survey of 76 plant species sampled from the shores of Van Mijenfjorden on Spitsbergen in the  
54      Svalbard archipelago, Väre et al. (1992) recorded no AM hyphae, vesicles or arbuscules in roots. The only  
55      evidence for the presence of AM fungi was a single spore, derived from a meadow below bird cliffs. This  
56      observation is counterintuitive: although situated in the High Arctic at c. 77–80 °N, Spitsbergen has a mild  
57      climate for its latitude, with the West Spitsbergen Current bringing warm water to the island's western shores all  
58      year round, and with approximate mean annual and summer air temperatures (2001–2015) of -3.3 °C and 4.8 °C,  
59      respectively (Isaksen et al. 2016). Given that the abundance of AM fungal structures in roots in polar ecosystems  
60      is positively associated with seasonal air temperatures (Upson et al. 2008) and that AM endophytes colonise  
61      roots in the colder Canadian High Arctic (Olsson et al. 2004; Allen et al. 2006; Ormsby et al. 2007), it seems  
62      plausible that the AM symbiosis is present on Spitsbergen. In support of this, a recent study used the polymerase  
63      chain reaction (PCR) to amplify AM fungal DNA from the roots of one *Saxifraga oppositifolia* and three  
64      *Ranunculus sulphureus* plants sampled from two locations in Longyearbyen on the island (Öpik et al. 2013).  
65      However, it is not presently known if the structural features of the symbiosis form in roots on Spitsbergen. We  
66      hence report a morphological study that examined the roots of likely host plant species sampled from 12  
67      locations on Spitsbergen for arbuscular mycorrhizas to determine if structures indicative of the symbiosis are  
68      present, and to determine if edaphic factors explain the abundance of the symbiosis on the island.

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71

72 **Materials and methods**

73 One hundred and two individuals of 13 plant species were collected in summer (late July–early September)  
74 2014, 2015 and 2016 from 12 sites on Spitsbergen in the Svalbard archipelago (Table 1). The 12 sites were  
75 located on the shores of Isfjorden and Kongsfjorden, 40 km and 150 km north of Van Mijenfjorden, respectively  
76 (Online Resource 1). Where possible, plants were collected from closed, undisturbed swards of vegetation, in  
77 which AM inoculum is more likely to develop and persist (Evans and Miller 1988). The plant species chosen  
78 were members of the Poaceae, Ranunculaceae and Compositae, and thus likely hosts for AM fungi. In order to  
79 facilitate identifications, which were performed using the flora of Lid and Lid (2005) and descriptions of  
80 vascular plants on Svalbard ([www.svalbardflora.no](http://www.svalbardflora.no)), flowering individuals of the majority of plants, and of all  
81 grasses, were collected. Mean summer air temperatures in 2001–2015 at three locations situated 1–18 km from  
82 the sampling sites ranged between 4.5 °C and 5.9 °C (Isaksen et al. 2016).

83 Individuals of each plant species were excavated with roots and attached soil and were placed into clean  
84 plastic bags, which were brought to the laboratory and kept at c. 4 °C. Within three days of sampling, the roots  
85 of each plant were removed from soil after tracing them back to the aboveground plant parts. The roots were  
86 initially cleaned in running water, were cleared in 10% KOH at 70 °C for 30 min and were then washed three  
87 times in water. Hydrogen peroxide (4%) was applied for 1–2 min. to roots that had not cleared sufficiently and  
88 was subsequently removed by washing several times in water. The roots were then acidified in 5% lactic acid at  
89 70 °C for 30 min and were stained with aniline blue (0.01%) in lactic acid, again at 70 °C for 30 min. The roots  
90 were then removed from the aniline blue solution, were blotted on tissue to remove excess stain, and were stored  
91 in 80% lactic acid. All of the roots obtained from each plant (200–530 mm) were mounted on glass slides in 80%  
92 lactic acid and were examined under UV fluorescence at 200–400 × magnification using an Olympus BX51  
93 microscope equipped with UPlanApo 20 × and 40 × objective lenses, a 100 W mercury short arc lamp and a UV  
94 fluorescence filter cube (U-MWU2, consisting of a BP 330–385 excitation filter, a DM 400 dichromatic mirror  
95 and an LP 420 emission filter; Olympus Life Science, Tokyo, Japan). The magnified intersections method of  
96 McGonigle et al. (1990) was used to determine the abundances of AM structures in roots. These were defined as  
97 either coarse AM endophyte with aseptate hyphae (5–10 µm diameter) and branched arbuscules with or without  
98 the presence of vesicles, or fine AM endophyte, with arbuscules and thin (1–3 µm diameter) hyphae,  
99 occasionally branching into fan-like patterns (Orchard et al. 2017). Between 100 and 150 evenly-spaced  
100 intersections were scored per individual root system (McGonigle et al. 1990).

101

102 *Soil chemistry*

103 Between four and 11 replicate soil samples from each site were analysed for pH and organic matter, soil  
104 moisture, and total carbon (C), nitrogen (N) and P concentrations. Soil pH was measured by adding double the  
105 volume of distilled water to each soil sample and recording pH using a glass electrode (Hanna Instruments,  
106 Leighton Buzzard, U.K.). Soil moisture was measured by heating 1–3 g of fresh soil to 105 °C for >3 h, prior to  
107 weighing, and organic matter concentrations were measured by heating the dried soil to 550 °C for 2 h, also prior  
108 to weighing (Allen 1989). Following grinding and sieving (2 mm), dried soil samples (2–9 mg) were placed into  
109 tin capsules and analysed for total C and N using a Costech ECS 4010 elemental analyser (Costech Analytical  
110 Technologies Inc., Valencia, USA). Total P was analysed for by digesting a sub-sample (0.36 g) of dried soil in  
111 4.4 ml of a sulphuric acid and hydrogen peroxide mixture with lithium sulphate and selenium powder, followed

112 by heating for 2 h, dilution and then colorimetric determination using molybdenum blue chemistry on a SEAL  
113 AQ2 discrete analyser (SEAL Analytical Ltd., Southampton, UK).

114

### 115 *Statistical analyses*

116 Associations between edaphic factors and the abundances of AM structures in roots were tested with principal  
117 component analysis (PCA), using the mean values of the measured response and predictor variables at each  
118 sampling site. All edaphic variables were zero-skew transformed and scaled from -1 to 1. In order to circumvent  
119 multiple testing and covariation between edaphic variables, a site-based PCA was conducted on the edaphic  
120 variables using the vegan package in R (Oksanen et al. 2017). Associations between the PCA axes and the  
121 individual edaphic variables were estimated using Kendall's Tau correlations, and those between the first two  
122 PCA axis scores and the abundance of each AM structure were tested using Pearson's product moment  
123 correlations. Air temperature measurements were not available at sufficient resolution to include them in the  
124 PCA.

125

## 126 **Results**

127 AM colonisation was recorded in the roots of 41 of the 102 plants sampled (Table 1). Eleven species of plant  
128 were found to be colonised by AM endophytes at 10 of the 12 sites (Table 1). Colonisation was particularly  
129 frequent in roots sampled from dense grass swards in Colesdalen and Adventdalen and at Ossian Sars, and was  
130 absent from roots sampled from one site at Kapp Linné and one in Longyearbyen (Table 1). The structures  
131 observed in roots were typical of AM colonisation, with characteristic infection units being formed consisting of  
132 vesicles, coarse AM hyphae with angular projections, entry points in root hairs and occasional arbuscules  
133 (Online Resource 2). Coarse AM hyphae and vesicles were recorded in the roots of 31 and 29 plants, in which  
134 they colonised 1.0–30.7% and 0.8–18.3% of root length, respectively (Table 1). Arbuscules occupied 0.7–11.9%  
135 of root length in 15 plants, all but two of which were grasses (Table 1). The hyphae of fine endophytes were also  
136 recorded in the roots of 28 plants, in which they colonised 0.7–12.8% of root length (Table 1).

137 In the PCA on the associations between AM abundances and edaphic factors (Table 2), the first two  
138 axes summarized 79% of the total variation in edaphic variables (PC1=61%, PC2=18%). Soil moisture and C, N,  
139 P and total organic matter concentrations were correlated with the first ordination axis ( $P<0.01$ ), while soil pH  
140 and C:N ratio were associated with the second axis ( $P<0.005$ ). The abundance of each individual AM structure,  
141 and of total AM colonisation, was not significantly correlated with the edaphic PCA axis scores (all  $P>0.07$ , data  
142 not shown).

143

## 144 **Discussion**

145 The observations reported here show that structures indicative of the AM symbiosis occur in the roots of grasses  
146 and forbs at several locations on the west coast of Spitsbergen. Whilst molecular analyses have PCR-amplified  
147 the DNA of *Glomus*, *Archaeospora* and *Claroideoglomus* spp. from the roots of plants in Longyearbyen (Öpik et  
148 al. 2013), our observations indicate that the symbiosis occurs more widely on the island, and that significant  
149 lengths of root can be occupied by AM structures in colonised plants. In common with observations at other  
150 locations in the High Arctic (Olsson et al. 2004; Allen et al. 2006; Ormsby et al. 2007), the occurrence in roots  
151 of arbuscules, the point at which P and other elements are transferred from the fungal symbiont to plant tissues

152 (Smith and Read 2008), suggests that AM symbioses have the capacity to influence plant nutrient acquisition on  
153 Spitsbergen.

154 Several of the plant species that were found to be colonised by AM endophytes in the present study  
155 (*Deschampsia alpina*, *Trisetum spicatum*, *Ranunculus sulphureus*, *R. pygmaeus*, *R. nivalis* and *Taraxacum*  
156 *arcticum*) were reported to be non-mycorrhizal by Väre et al. (1992). What might explain the previously reported  
157 absence of the AM symbiosis from the roots of these plant species on Spitsbergen? One possibility is that the  
158 sampling employed by Väre et al. (1992) was not extensive enough to have detected AM symbionts in roots.  
159 This is unlikely, since a total of 205 root samples – twice the number of samples gathered here – collected from  
160 17 sites distributed over a distance of >100 km on the shores of Van Mijenfjorden were examined for AM  
161 structures by Väre et al. (1992). Although many of these samples were of plant species belonging to typically  
162 non-mycorrhizal families, which increase in abundance at high latitudes (Newsham et al. 2009), AM  
163 colonisation was found to be absent from the roots of 54 members of the Poaceae and 15 members of the  
164 Ranunculaceae (Väre et al. 1992), families that were found to be colonised by AM endophytes in the present  
165 study.

166 Contrary to previous reports (e.g. Liu et al. 2017), edaphic factors had no apparent influence on the  
167 abundances of AM structures in the present study. It thus seems unlikely that differences in soil chemistry  
168 between the sites sampled by Väre et al. (1992) and those studied here explain the discrepancies between the two  
169 studies. Differences in the plant community types sampled might also explain these discrepancies, but the  
170 communities from which plants were sampled by Väre et al. (1992) included dwarf shrub heaths, meadows and  
171 grass swards, and were thus similar to those studied here. The previously reported absence of AM structures  
172 from plant roots on Spitsbergen may have been owing to Väre et al. (1992) gathering some roots at the start of  
173 the growing season on the island (early June), when the abundances of AM structures in roots may have been  
174 low (Mullen and Schmidt 1993). However, Väre et al. (1992) also sampled roots up to the middle of the growing  
175 season (mid August), by which time suitable conditions for AM colonisation of roots should have occurred, and  
176 so this explanation can most probably also be discounted.

177 It is possible that increasing surface air temperatures on Spitsbergen since 1969 may have led to more  
178 abundant AM colonisation of roots in recent decades, explaining the difference between the results of Väre et al.  
179 (1992) and those reported here. Assuming a rise in mean annual air temperature on the island of c. 2.0 °C since  
180 the early 1970s (Isaksen et al. 2016), and 0.6–3.0% increases in the percentage of root length occupied by AM  
181 structures per degree Celsius increase in air temperature along transects through polar habitats (Upson et al.  
182 2008), then up to 6% increases in the abundances of AM endophytes in roots might be anticipated over this  
183 period. Modest increases in root lengths occupied by AM structures owing to climate warming since the late  
184 1960s are, however, unlikely to explain the differences between the findings of the two studies. A more plausible  
185 explanation is that some of the sites sampled here had more favourable microclimates than those on the shores of  
186 Van Mijenfjorden: although ambient air temperatures around this fjord are only c. 1 °C lower than those around  
187 Isfjorden and Kongsfjorden (Pryzbylak 1992), it is notable that AM colonisation was particularly abundant in the  
188 present study at Colesdalen, which has a high concentration of thermophilous plant species (Alsos et al. 2004),  
189 and at Ossian Sars, which short-term meteorological data indicate is one of the warmest locations in  
190 Kongsfjorden (Joly et al. 2010). Microclimatic data at sufficient resolution to make meaningful predictions of  
191 soil biological activity at the sites studied here are unavailable, and we were hence unable to include air or soil

192 temperatures as predictors in our statistical analyses. However, we propose that by sampling from locations with  
193 favourable microclimates, and particularly from dense swards of vegetation, in which AM inoculum is more  
194 likely to persist (Evans and Miller 1988), we would have increased the likelihood of recording AM symbionts in  
195 roots, perhaps explaining the disparity between the results of the present study and those of Väre et al. (1992).

196 We conclude that structures formed by AM fungi are indeed present in the roots of plants on  
197 Spitsbergen, expanding the known range of the symbiosis in the High Arctic (*c.f.* Olsson et al. 2004; Allen et al.  
198 2006; Ormsby et al. 2007; Newsham et al. 2009). Further studies should examine whether microclimate does  
199 influence the abundances of AM structures in roots on Spitsbergen, as it appears to at other locations in the High  
200 Arctic (Allen et al. 2006), and whether the AM symbiosis affects plant performance on the island, for example if  
201 it enhances drought tolerance or nutrient acquisition from cold soils (Tibbett and Cairney 2007; Helgason and  
202 Fitter 2009). Further research should also determine whether the roots of other plant species on Spitsbergen are  
203 colonised by AM fungi, focusing on the remaining members of the families studied here and members of  
204 speciose families present on the island that are usually AM at lower latitudes, such as the Saxifragaceae and  
205 Rosaceae (Wang and Qui 2006).

206

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217

- 218 **References**
- 219 Allen N, Nordlander M, McGonigle T, Basinger J, Kaminskyj S (2006) Arbuscular mycorrhizae on Axel  
220 Heiberg Island (80 °N) and at Saskatoon (52 °N) Canada. *Can J Bot* 84:1094–1100
- 221
- 222 Allen SE (1989) Chemical Analysis of Ecological Materials, 2<sup>nd</sup> edn. Blackwell, Oxford, p 368
- 223
- 224 Alsos IG, Westergaard K, Lund L, Sandbakk BE (2004) The flora of Colesdalen, Svalbard. *Blyttia* 62:142–151
- 225
- 226 Evans DG, Miller MH (1988) Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of  
227 nutrient absorption in maize. I. Causal relations. *New Phytol* 110:67–74
- 228
- 229 Helgason T, Fitter AH (2009) Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi  
230 (phylum Glomeromycota). *J Exp Bot* 60:2465–2480
- 231
- 232 Isaksen K, Nordli Ø, Førland EJ, Łupikasza E, Eastwood S, Niedźwiedź T (2016) Recent warming on  
233 Spitsbergen – influence of atmospheric circulation and sea ice cover. *J Geophys Res Atmos* 121:11913–11931
- 234
- 235 Joly D, Nilssen L, Brossard T, Elvebakken A (2010) Plants as bioindicator for temperature interpolation purposes:  
236 analyzing spatial correlation between botany based index of thermophily and integrated temperature  
237 characteristics. *Ecol Indicators* 10:990–998
- 238
- 239 Lid J, Lid DT (2005) Norsk flora, 7<sup>th</sup> edn. Det Norske Samlaget, Oslo, p 1230
- 240
- 241 Liu M, Zheng R, Bai S, Bai Y, Wang J (2017) Slope aspect influences arbuscular mycorrhizal fungus  
242 communities in arid ecosystems of Daqingshan Mountains, Inner Mongolia, North China. *Mycorrhiza* 27:189–  
243 200
- 244
- 245 McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swann JA (1990) A new method which gives an objective  
246 measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 155:495–501
- 247
- 248 Mullen RB, Schmidt SK (1993) Mycorrhizal infection, phosphorus uptake, and phenology in *Ranunculus*  
249 *adoneus*: implications for the functioning of mycorrhizae in alpine systems. *Oecologia* 94:229–234
- 250
- 251 Newsham KK, Upson R, Read DJ (2009) Mycorrhizas and dark septate root endophytes in cold regions. *Fungal*  
252 *Ecol* 2:10–20
- 253
- 254 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL,  
255 Solymos P, Stevens MHH, Szoecs E, Wagner H (2017) Vegan: Community Ecology Package. R package  
256 version 2.4-3. <https://CRAN.R-project.org/package=vegan>
- 257

- 258 Olsson PA, Eriksen B, Dahlberg A (2004) Colonization by arbuscular mycorrhizal and fine endophytic fungi in  
259 herbaceous vegetation in the Canadian High Arctic. Can J Bot 82:1547–1556
- 260
- 261 Öpik M, Zobel M, Cantero JJ, Davison J, Facelli JM, Hiiesalu I, Jairus T, Kalwij JM, Koorem K, Leal ME, Liira  
262 J, Metsis M, Neshataeva V, Paal J, Phosri C, Reier U, Thiery O, Vasar M, Moora M (2013) Global sampling of  
263 plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza 23:411–430
- 264
- 265 Orchard S, Hilton S, Bending GD, Dickie IA, Standish, R.J., Gleeson D, Jeffery RP, Powell 437 JR, Walker C,  
266 Bass D, Monk J, Simonin A, Ryan M H (2017) Fine endophytes (*Glomus tenue*) are related to Mucoromycotina,  
267 not Glomeromycota. New Phytol 213: 481–486
- 268
- 269 Ormsby A, Hodson E, Li Y, Basinger J, Kaminskyj S (2007) Quantitation of endorhizal fungi in High Arctic  
270 tundra ecosystems through space and time: the value of herbarium archives. Can J Bot 85:599–606
- 271
- 272 Pryzbylak R (1992) Spatial differentiation of air temperature and humidity on western coast of Spitsbergen in  
273 1979–1983. Polish Polar Res 13:113–129
- 274
- 275 Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47:376–391
- 276
- 277 Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd edn. Academic, London
- 278
- 279 Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I,  
280 Gryganskyi A, James TY, O'Donnell K, Roberson RW, Taylor TN, Uehling J, Vilgalys R, White MM, Stajich  
281 JE (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data.  
282 Mycologia 108:1028–1046
- 283
- 284 Tibbett M, Cairney JWG (2007) The cooler side of mycorrhizas: their occurrence and functioning at low  
285 temperatures. Can J Bot 85:51–62
- 286
- 287 Upson R, Newsham KK, Read DJ (2008) Root-fungal associations of *Colobanthus quitensis* and *Deschampsia*  
288 *antarctica* in the maritime and sub-Antarctic. Arct Antarct Alp Res 40:592–599
- 289
- 290 Väre H, Vestberg M, Eurola, S (1992) Mycorrhiza and root associated fungi in Spitsbergen. Mycorrhiza 1:93–  
291 104
- 292
- 293 Wang B, Qui Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza  
294 16:299–363

**Table 1** Details of the 12 sampling sites and the frequencies of AM colonisation in roots

| Sampling site <sup>a</sup> | Latitude and longitude      | Plant community                                     | Plant species   | Plants sampled (n) | Coarse AM hyphae <sup>b</sup> | Vesicles <sup>b</sup>   | Arbuscules <sup>b</sup> | Fine endophyte hyphae <sup>b</sup> |
|----------------------------|-----------------------------|---|---|--------------------|-------------------------------|-------------------------|-------------------------|------------------------------------|
| Adventdalén                | 78°10.271'N,<br>16°00.668'E | Dense grass<br>swards<br>interspersed with<br>forbs | <i>Alopecurus ovatus</i> Knapp                                      | 7                  | 2 (22.9, 6.6)                 | 2 (18.3, 6.2)           | 1 (11.9)                | 1 (0.9)                            |
|                            |                             |   | <i>Calamagrostis neglecta</i> (Ehrh.) P. Gaertn., B. Mey. & Scherb. | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Festuca rubra</i> L. ssp. <i>richardsonii</i> (Hoop.) Hultén     | 5                  | 1 (14.7)                      | 1 (6.2)                 | 1 (2.3)                 | 1 (3.8)                            |
|                            |                             |   | <i>Poa arctica</i> R. Br. / <i>P. pratensis</i> L. <sup>c</sup>     | 2                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Poa arctica</i> ssp. <i>arctica</i> R. Br.                       | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Poa pratensis</i> L.   | 2                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Ranunculus sulphureus</i> Sol.                                   | 4                  | 3 (21.8, 10.0, 3.4)           | 3 (11.9, 8.2, 3.4)      | 0                       | 1 (11.9)                           |
| Colesdalén                 | 78°06.468'N,<br>15°04.729'E | Dense grass<br>swards<br>interspersed with<br>forbs | <i>Alopecurus ovatus</i>  | 2                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Festuca rubra</i> ssp. <i>richardsonii</i>                       | 1                  | 1 (8.6)                       | 1 (2.9)                 | 1 (3.8)                 | 0                                  |
|                            |                             |   | <i>Ranunculus pygmaeus</i> L.                                       | 1                  | 1 (22.5)                      | 1 (12.5)                | 0                       | 1 (1.7)                            |
|                            |                             |   | <i>Ranunculus sulphureus</i>  | 4                  | 3 (4.2, 3.3, 1.7)             | 3 (2.5, 2.4, 0.8)       | 1 (0.8)                 | 2 (4.2, 2.5)                       |
|                            |                             |   | <i>Ranunculus nivalis</i> L.  | 2                  | 2 (19.2, 17.7)                | 2 (15.4, 9.7)           | 0                       | 0                                  |
|                            |                             |   | <i>Taraxacum arcticum</i> (Trautv.) Dahlst.                         | 1                  | 1 (7.7)                       | 1 (4.3)                 | 0                       | 1 (1.7)                            |
| Endalen 1                  | 78°11.035'N,<br>15°45.557'E | Dwarf-shrub<br>heath tundra                         | <i>Alopecurus ovatus</i>  | 4                  | 1 (3.0)                       | 1 (3.0)                 | 0                       | 0                                  |
|                            |                             |   | <i>Festuca rubra</i> ssp. <i>richardsonii</i>                       | 2                  | 0                             | 0                       | 0                       | 1 (3.0)                            |
|                            |                             |   | <i>Poa arctica</i> / <i>P. pratensis</i> <sup>c</sup>               | 3                  | 2 (16.5, 12.4)                | 2 (9.5, 3.1)            | 2 (0.8, 0.7)            | 1 (1.6)                            |
|                            |                             |   | <i>Poa arctica</i> ssp. <i>arctica</i>                              | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Ranunculus sulphureus</i>  | 11                 | 3 (12.0, 5.7, 5.1)            | 3 (5.2, 4.4)            | 0                       | 4 (4.4, 4.1, 3.0, 2.0)             |
| Endalen 2                  | 78°11.180'N,<br>15°45.557'E | Dwarf-shrub<br>heath tundra                         | <i>Alopecurus ovatus</i>  | 2                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Ranunculus sulphureus</i>  | 4                  | 1 (7.6)                       | 1 (5.9)                 | 0                       | 1 (4.0)                            |
| Kapp Linné 1               | 78°03.614'N,<br>13°38.293'E | Dwarf-shrub<br>heath tundra                         | <i>Deschampsia alpina</i> (L.) Roem. & Schult.                      | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Poa arctica</i> / <i>P. pratensis</i> <sup>c</sup>               | 3                  | 0                             | 0                       | 0                       | 1 (0.7)                            |
| Kapp Linné 2               | 78°03.569'N,<br>13°38.554'E | Dwarf-shrub<br>heath tundra                         | <i>Poa arctica</i> / <i>P. pratensis</i> <sup>c</sup>               | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Poa arctica</i> ssp. <i>arctica</i>                              | 2                  | 0                             | 0                       | 0                       | 1 (1.0)                            |
| Kapp Linné 3               | 78°03.532'N,<br>13°39.075'E | Dwarf-shrub<br>heath tundra                         | <i>Deschampsia alpina</i>   | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Poa arctica</i> / <i>P. pratensis</i> <sup>c</sup>               | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Poa arctica</i> ssp. <i>arctica</i>                              | 1                  | 0                             | 0                       | 0                       | 0                                  |
| Kapp Linné 4               | 78°04.484'N,<br>13°45.508'E | Dwarf-shrub<br>heath tundra                         | <i>Deschampsia alpina</i>   | 2                  | 1 (2.0)                       | 1 (1.0)                 | 0                       | 1 (1.0)                            |
|                            |                             |   | <i>Festuca rubra</i> ssp. <i>richardsonii</i>                       | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Poa arctica</i> / <i>P. pratensis</i> <sup>c</sup>               | 3                  | 0                             | 0                       | 0                       | 0                                  |
| Kvadehuken                 | 78°58.243'N,<br>11°28.521'E | Sparse grass<br>sward                               | <i>Festuca rubra</i> ssp. <i>richardsonii</i>                       | 4                  | 0                             | 0                       | 1 (5.2)                 | 1 (5.2)                            |
| Longyearbyen 1             | 78°13.148'N,<br>15°38.825'E | Moss wetland  | <i>Coptidium spitsbergense</i> (Hadač) Luferov & Prob.              | 4                  | 1 (3.0)                       | 0                       | 0                       | 0                                  |
| Longyearbyen 2             | 78°13.033'N,<br>15°39.114'E | Dwarf-shrub<br>heath tundra                         | <i>Alopecurus ovatus</i>  | 5                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Coptidium spitsbergense</i>                                      | 1                  | 0                             | 0                       | 0                       | 0                                  |
|                            |                             |   | <i>Ranunculus sulphureus</i>  | 1                  | 0                             | 0                       | 0                       | 0                                  |
| Ossian Sars                | 78°55.795'N,<br>12°26.680'E | Dense grass<br>swards<br>interspersed with<br>forbs | <i>Festuca rubra</i> ssp. <i>richardsonii</i>                       | 5                  | 4 (16.8, 14.2, 13.4, 8.4)     | 4 (10.4, 9.8, 6.7, 6.5) | 4 (5.9, 2.5, 1.1, 0.7)  | 4 (9.2, 5.8, 1.1, 0.7)             |
|                            |                             |   | <i>Poa arctica</i> / <i>P. pratensis</i> <sup>c</sup>               | 1                  | 1 (1.0)                       | 1 (1.0)                 | 1 (2.5)                 | 1 (10.0)                           |
|                            |                             |   | <i>Poa arctica</i> ssp. <i>arctica</i>                              | 2                  | 1 (1.1)                       | 1 (1.1)                 | 1 (2.4)                 | 2 (6.4, 1.1)                       |
|                            |                             |   | <i>Ranunculus sulphureus</i>  | 2                  | 1 (12.0)                      | 1 (7.7)                 | 1 (0.7)                 | 2 (12.8, 5.9)                      |
|                            |                             |   | <i>Trisetum spicatum</i> (L.) K. Richt.                             | 1                  | 1 (30.7)                      | 1 (17.3)                | 1 (2.7)                 | 1 (1.7)                            |

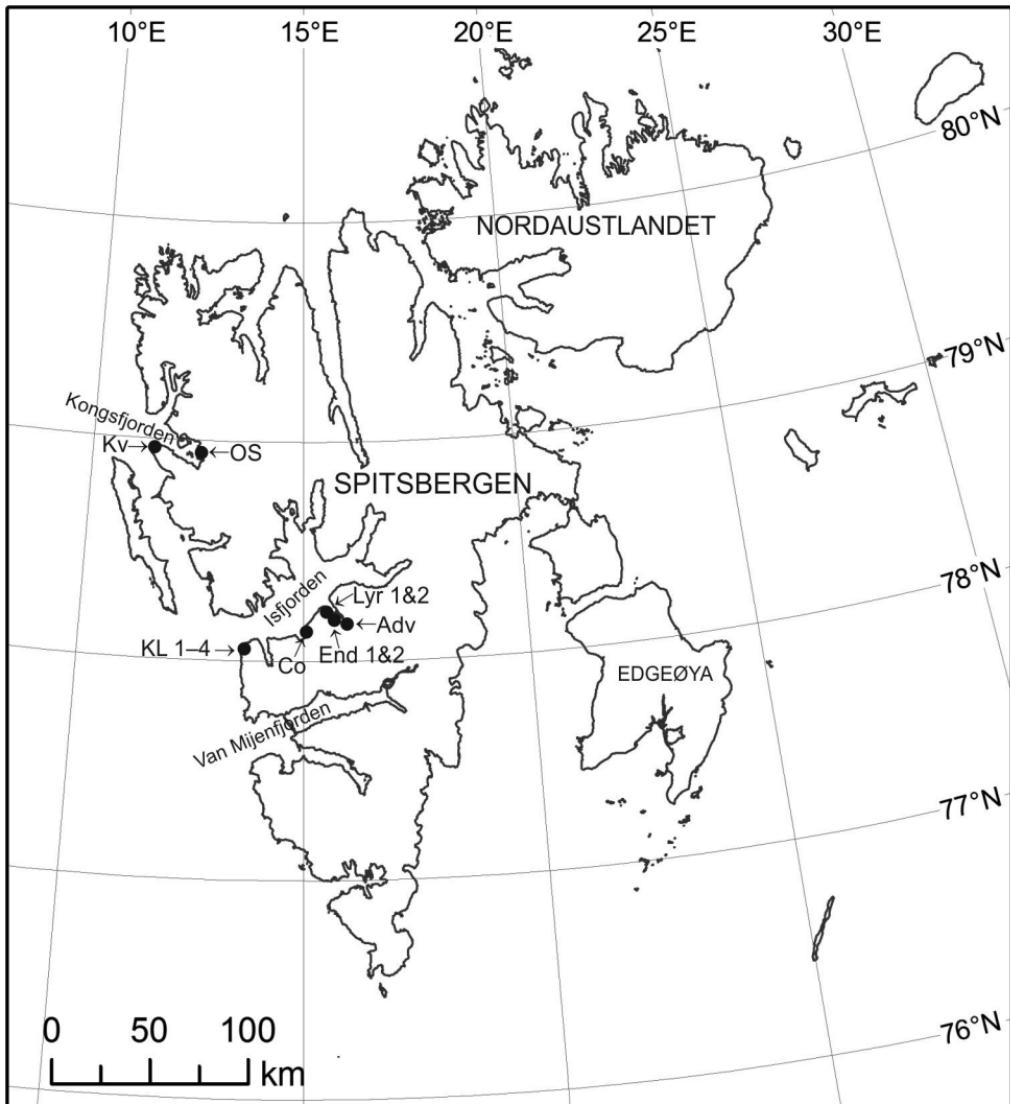
<sup>a</sup>See Online Resource 1 for a map showing the locations of the sampling sites; <sup>b</sup>values shown are the numbers of colonised plants, followed in parentheses by the mean percentage root length occupied by each AM structure in the roots of each colonised plant;

<sup>c</sup>viviparous plants. The viviparous plants of the *Poa arctica*-*P. pratensis* complex (except for *P. pratensis* ssp. *colpodea*) span the range of morphological variation between *P. arctica* ssp. *arctica* and *P. pratensis* ssp. *alpigena*, possibly arising from hybridization between the two species, and are thus morphologically indistinguishable (R. Elven, pers. comm.).

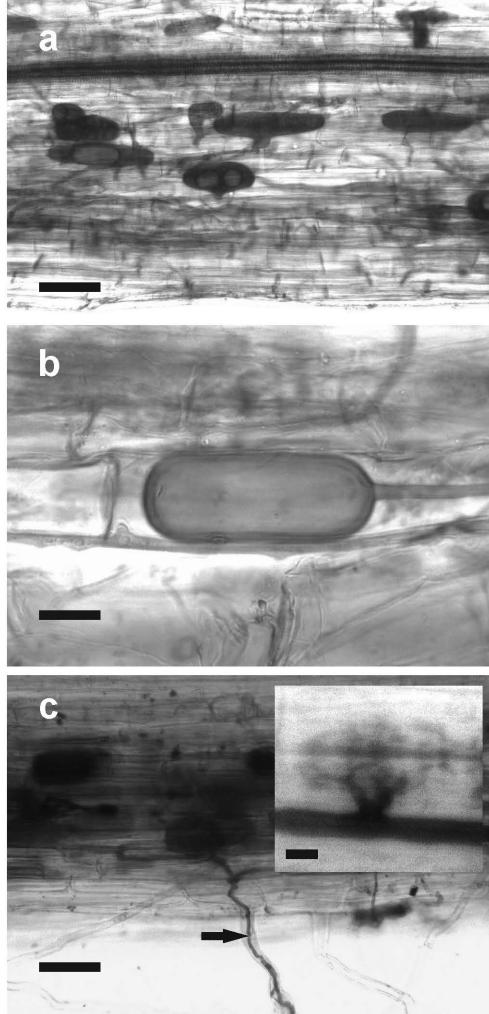
**Table 2** Edaphic factors at the 12 sampling sites

| Site <sup>a</sup> | Edaphic factor <sup>a</sup> |             |              |                         |                |                    |  |
|-------------------|-----------------------------|-------------|--------------|-------------------------|----------------|--------------------|--|
|                   | pH                          | Carbon (%)  | Nitrogen (%) | Carbon : nitrogen ratio | Phosphorus (%) | Organic matter (%) | Moisture (g water per 100 g oven-dried soil) |
| Adventdalen       | 6.0 ± 0.3                   | 25.1 ± 5.5  | 1.0 ± 0.1    | 25.4 ± 1.9              | 0.10 ± 0.03    | 26.7 ± 16.7        | 44.4 ± 11.6                                  |
| Colesdalen        | 5.3 ± 0.2                   | 6.5 ± 6.1   | 0.5 ± 0.4    | 12.3 ± 3.3              | 0.09 ± 0.01    | 9.3 ± 2.1          | 29.2 ± 13.2                                  |
| Endalen 1         | 5.4 ± 0.5                   | 11.5 ± 5.7  | 0.5 ± 0.2    | 23.9 ± 5.7              | 0.04 ± 0.02    | 10.0 ± 4.6         | 34.4 ± 9.1                                   |
| Endalen 2         | 6.5 ± 0.4                   | 32.4 ± 13.2 | 2.4 ± 3.2    | 13.3 ± 6.8              | 0.08 ± 0.01    | 27.2 ± 12.7        | 56.8 ± 17.9                                  |
| Kapp Linné 1      | 6.9 ± 0.2                   | 6.6 ± 1.6   | 0.3 ± 0.2    | 20.6 ± 1.9              | 0.03 ± 0.01    | 4.0 ± 0.2          | 30.6 ± 17.8                                  |
| Kapp Linné 2      | 7.3 ± 0.4                   | 6.8 ± 2.2   | 0.4 ± 0.2    | 16.5 ± 0.12             | 0.06 ± 0.01    | 10.8 ± 4.6         | 25.4 ± 15.7                                  |
| Kapp Linné 3      | 6.8 ± 0.3                   | 12.1 ± 7.3  | 1.0 ± 0.4    | 12.5 ± 1.2              | 0.09 ± 0.06    | 11.3 ± 12.4        | 60.4 ± 6.9                                   |
| Kapp Linné 4      | 6.6                         | 8.1         | 1.3          | 6.2                     | m.d.           | m.d.               | m.d.   |
| Kvadehuken        | 6.7 ± 0.3                   | 28.7 ± 7.7  | 1.7 ± 0.4    | 16.8 ± 2.4              | 0.24 ± 0.14    | 46.5 ± 17.2        | 63.6 ± 10.1                                  |
| Longyearbyen 1    | 4.7 ± 0.3                   | 27.7 ± 2.4  | 1.0 ± 0.2    | 27.5 ± 6.8              | 0.13 ± 0.02    | 58.1 ± 5.1         | 80.4 ± 4.4                                   |
| Longyearbyen 2    | 6.4 ± 0.2                   | 17.0 ± 3.5  | 0.8 ± 0.1    | 22.6 ± 2.5              | 0.03 ± 0.01    | 15.6 ± 4.3         | 53.8 ± 9.1                                   |
| Ossian Sars       | 6.2 ± 0.4                   | 32.1 ± 15.9 | 1.9 ± 0.8    | 16.9 ± 8.2              | 0.13 ± 0.03    | 65.5 ± 28.6        | 59.8 ± 13.3                                  |

<sup>a</sup> all values except those for Kapp Linné 4 (which are single measurements) are means ( $n = 4\text{--}11$ ) ± standard deviation; m.d.; missing data.



**Online Resource 1** Map of the Svalbard archipelago showing the locations of the 12 sampling sites on Spitsbergen. Abbreviations: Kv, Kvadehuken; OS, Ossian Sars; KL, Kapp Linné; Co, Colesdalen; Lyr, Longyearbyen; End, Endalen; Adv, Adventdal.



**Online Resource 2** Light micrographs of AM structures in plant roots from Spitsbergen.  
**a** Vesicles in a *Ranunculus sulphureus* root. **b** An intracellular vesicle in a root epidermal cell of *Alopecurus ovatus*. **c** An entry point of coarse AM hyphae (arrowed) in a root hair of *A. ovatus*. Insert in **c** shows an arbuscule formed in a root cortical cell of *A. ovatus*. The image is a composite of five successive images stacked using Helicon Focus v. 6.7.1 with automatic contrast optimization. Scale bars in **a–c** are 100, 30 and 60 µm respectively and that in the insert in **c** is 5 µm.