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Abstract

Truelove Lowland on Devon Island, Nunavut (75°N), has long been investigated for its flora, fauna, and microbiota. Unlike ectomycorrhizae, endomycorrhizal interactions have been described as sparse or absent in this High Arctic environment. To probe this observation, samples of roots and associated soils (55 plants in total) from 10 genera in 9 families were collected during July 2006. Fungi growing within these roots were visualized using our high-sensitivity lactofuchsin epifluorescence method. Fungal colonization within plant roots (collectively, endorhizal fungi) was assessed with our quantitative microintersect method. Of the 3988 intersections assessed at 400× total magnification, only 154 lacked fungi. Most colonization was by septate endophytes (average abundance 66%, range 13–100%), and fine endophytes (average abundance 48%, range 0–100%). Endorhizal morphology in *Dryas* and *Saxifraga* roots typically consisted of thin extraradical hyphae that formed a sheath and grew between and within root cortical cells, resembling ericoid or ectendomycorrhizae. Soil in which the Truelove plants had grown, which had been stored at −20 °C, was planted with wheat seeds. After 10 weeks, fungal colonization of these roots was 35–100%. Endorhizal fungi are typically present in roots of plants living on Devon Island tundra.

Introduction

Plant-fungus interactions are likely to have been a necessary prerequisite for plant colonization of land almost 450 million years ago (Taylor et al., 1995; Malloch et al., 1980; Krings et al., 2007), and today fungi are associated with most terrestrial plant species. These symbiotic relationships include mycorrhizae, wherein plants trade sugars produced by photosynthesis for mineral nutrients extracted from the soil by fungi (Smith and Read, 1997), and fungal endophytes that live asymptomatically within roots or aerial parts of plants and can confer habitat-adapted tolerance to extreme environments (Rodriguez et al., 2008). Extreme environments support few individuals or have low biodiversity due to their physical (for example, cold) and/or chemical (for example, low nutrient) characteristics.

The importance of mycorrhizal fungi is well documented for temperate and tropical plant communities (Smith and Read, 1997; Olsson et al., 2004), as is the importance of endophytic fungi (Rodriguez et al., 2009). Truelove Lowland, ca. 74°N on Devon Island, Nunavut (NU), is an Arctic oasis (Bledsoe et al., 1990) that is relatively rich in biological diversity and vascular plant flora and so may be an ideal location to evaluate fungus-plant root associations. Previous research, however, has suggested that arbuscular mycorrhizae (AM) symbioses were rare or even absent under Arctic conditions (Read and Haselwandter, 1981; Bledsoe et al., 1990; Kohn and Stasovski, 1990; Gardes and Dahlberg, 1996; Cripps and Eddington, 2005; Kytoviita, 2005). For example, AM interactions as shown by the presence of arbuscules were not found by Bledsoe et al. (1990) at Truelove, despite considerable effort. Nevertheless, we have previously found abundant and diverse fungus-plant root interactions including AM, fine endophytes (FE), and septate endophytes (SE), collectively called endorhizal fungi, in plants collected from tundra sites across the Canadian High Arctic (Allen et al., 2006; Ormsby et al., 2007; Hodson et al., 2009; Walker et al., 2010). Our studies used high-sensitivity confocal fluorescence imaging of lactofuchsin-stained roots (Kaminskyj, 2008), which had not been available previously. Given the opportunity to assess specimens collected on Truelove Lowland in 2006 for the presence of endorhizal fungi, we revisited this question.

Methods

SITE LOCATION AND SAMPLE COLLECTION

The sampling site for this study was Truelove Lowland, ca. 75°N, 84°W, on the northern coast of Devon Island, in the Canadian Arctic Archipelago (Fig. 1). Truelove Lowland vegetation is typical of Arctic tundra. Details of individual collection sites are given in Table 1.

Plants were collected during July 2006, and all were in flower at the time. Roots and flowers were preserved in buffered formalin, and stored at 4 °C (Allen et al., 2006). Soil samples associated with these roots were stored in Whirlpak® bags at −20 °C. In 2007, plants were identified using Porsild (1964). For each specimen, roots were prepared and soil samples were planted as described below.

ROOT PREPARATION AND MICROSCOPY/IMAGING

Root preparation, imaging, and quantification methods are described in Ormsby et al. (2007) and Kaminskyj (2008). Roots were cleared by autoclaving for 20 min in 10% KOH, which was
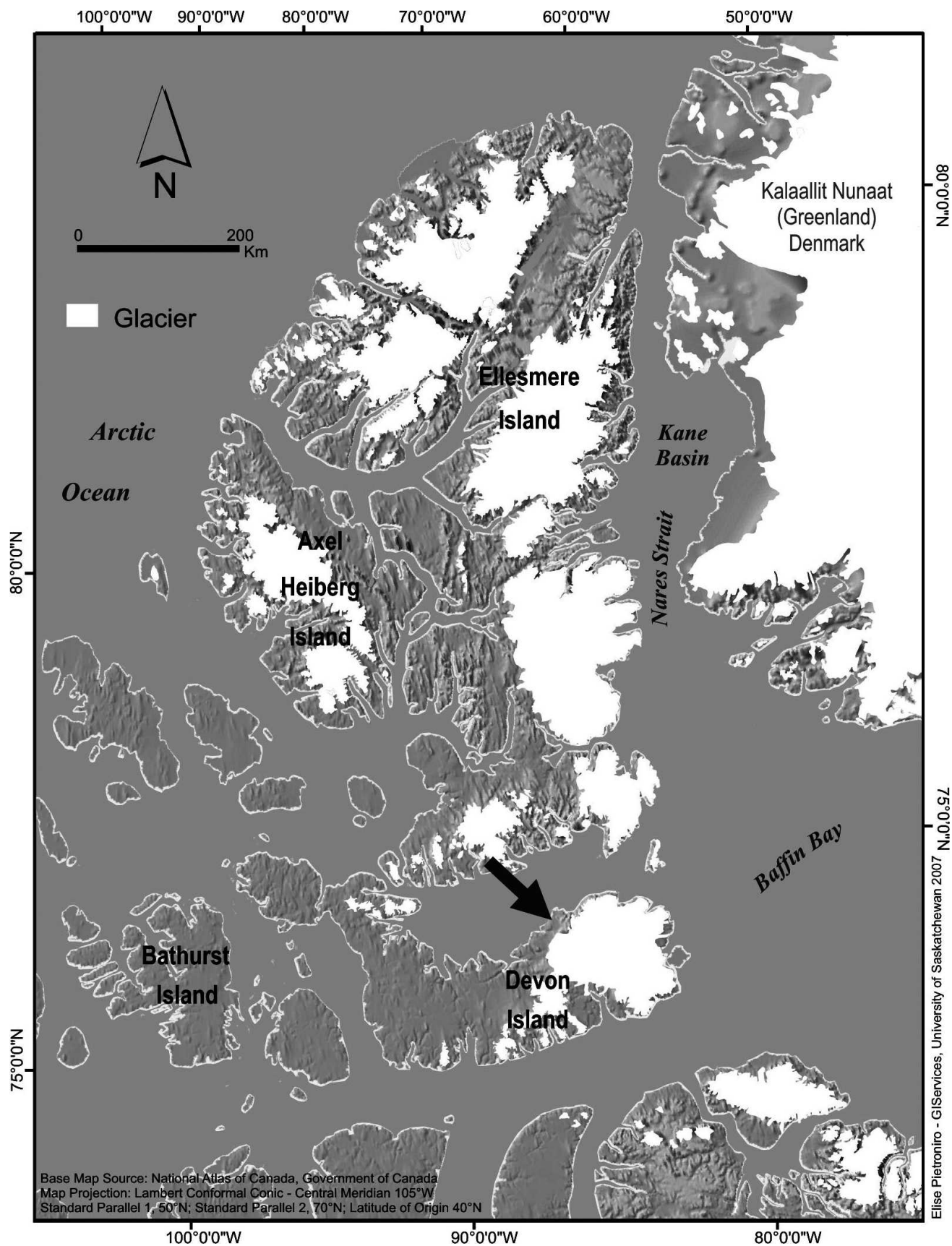
FIGURE 1. Location of Truelove Lowland (arrow) on Devon Island, Nunavut, Canada.

TABLE 1

Sampling site locations, plant genera, and families.

	Latitude (°N)	Longitude (°W)	Altitude (m)	Number of plants
Cassiope tetragona D.Don (Ericaceae)	75°40′19″	84°34′57″	16.3	1
Cerastium L. sp. (Caryophyllaceae)	75°40′27″	84°35′02″	15.1	2
Dryas integrifolia M. Vahl. (Rosaceae)	75°40′51″	84°38′36″	3.8	1
D. integrifolia	75°40′47″	84°31′03″	36.2	1
D. integrifolia	75°40′38″	84°31′40″	25.1	1
D. integrifolia	75°40′38″	84°31′22″	34.4	1
D. integrifolia	75°40′19″	84°34′57″	16.3	1
Epilobium latifolium L. (Onagraceae)	75°38′13″	84°27′40″	20.1	6
Eriophorum L. sp. (Cyperaceae)	75°40′47″	84°31′03″	36.2	1
Eriophorum sp.	75°40′37″	84°36′07″	12.9	2
Melandrium Roehl. sp. (Caryophyllaceae)	75°40′50″	84°38′42″	−2.4	2
Oxyria digyna (L.) Hill (Polygonaceae)	75°40′10″	84°34′42″	14.9	2
Papaver radicatum Rottb. (Papaveraceae)	75°40′51″	84°38′32″	1.5	1
P. radicatum	75°40′38″	84°31′40″	25.1	1
P. radicatum	75°40′38″	84°31′22″	34.4	1
P. radicatum	75°40′26″	84°34′58″	12.4	1
P. radicatum	75°40′21″	84°35′06″	14.4	2
Pedicularis arctica R. Br. (Scrophulariaceae)	75°41′18″	84°34′04″	21.7	2
P. arctica	75°40′47″	84°31′03″	36.2	2
P. arctica	75°40′37″	84°36′07″	12.9	2
P. capitata Adams	75°41′07″	84°34′18″	17.2	2
P. capitata	75°40′35″	84°30′39″	44.3	1
P. capitata	75°40′19″	84°34′57″	16.3	1
P. lanata Cham & Schlecht.	75°40′21″	84°35′06″	14.4	2
Saxifraga cernua L. (Saxifragaceae)	75°40′26″	84°34′59″	14.8	2
S. hirculus L.	75°40′51″	84°38′36″	3.8	1
S. hirculus	75°40′47″	84°31′03″	36.2	1
S. hirculus	75°40′38″	84°31′40″	25.1	2
S. hirculus	75°40′38″	84°31′22″	34.4	2
S. hirculus	75°40′37″	84°36′07″	12.9	2
S. hirculus	75°40′34″	84°37′09″	4.6	2
S. hirculus	75°40′00″	84°35′25″	9.8	2
S. nivalis L.	75°40′39″	84°30′37″	46.2	1
S. oppositifolia L.	75°40′38″	84°31′40″	46.2	1

then removed by two washes in 70% ethanol. Pigmented roots characteristic of some plant species were bleached in freshly prepared 8:1:1 distilled water: 28% ammonium hydroxide: 37% hydrogen peroxide. Bleaching was for 10–30 min at room temperature with gentle agitation, until roots were a pale cream color. Bleached roots were rinsed with two changes of distilled water. Roots were stained in 0.05% acid fuchsin in 85% lactic acid overnight at 47 °C. Roots were rinsed once in destaining solution (1:1:1 distilled water: 85% lactic acid: 100% glycerol) and then incubated in destaining solution at room temperature or 47 °C, for 4 h to overnight, until they were pale pink. Stained roots were mounted in polyvinyl alcohol glycerol medium, which was hardened overnight at 40 °C.

Roots were examined using confocal laser scanning microscopy (CLSM) for high-resolution imaging of endorhizal structures, and wide-field epifluorescence microscopy for endorhizal quantitation. CLSM imaging used a Zeiss META 510 laser-scanning microscope (http://www.zeiss.com) equipped with a 25× Plan NeoFluar N.A. 0.8 multi-immersion objective. CLSM image collection used a 543 nm HeNe1 laser, 9.9% intensity of a 25 mW beam, a HFT 488/543 beam splitter, and a 604–657 nm emission filter. Fluorescence and transmitted light images were collected simultaneously. Wide-field epifluorescence imaging for quantitation used a Zeiss Axioplan microscope equipped with a 40× N.A. 0.75 Plan Neofluar objective, an HBO50 light source, with a BP546 excitation filter, FT580 dichroic mirror, and LP590 emission filter. Images were captured using a Sensys CCD (http://www.roper.com) driven by MetaVue software (http://www.image1.com). Transmitted light was used to assess dark septate endophyte hyphae, which have melanized walls that do not fluoresce under these conditions.

Fungal colonization abundance was assessed using the multiple quantitation microintersect (MQM: Ormsby et al., 2007; Kaminskyj, 2008; Walker et al., 2010) method modified after McGonigle et al. (1990), for high spatial resolution quantitation of endorhizal colonization morphologies, hereafter referred to as endorhizal morphotypes. Intersections were defined by a graticule in the center of the field of view and were spaced one field-of-view apart. The entire length of shorter root systems was examined. Longer root systems were randomly subsampled as described by Allen et al. (2006). Intersections were inspected using 400× total magnification, focusing through the root section. Each intersection was assessed for several endorhizal morphotypes, including: 4- to 6-μm-wide aseptate hyphae characteristic of arbuscular mycorrhizae (AM); arbuscules and vesicles associated with AM hyphae; <1.5-μm-wide aseptate hyphae characteristic of fine endophytes (FE); arbuscules and vesicles associated with FE hyphae; 2.0- to 4.5-μm-wide septate endophyte hyphae (SE); dark septate endophyte (DSE) hyphae; clamp connections; microsclerotia; and spores. Total colonization was assessed using the number of intersections that lacked fungi, since many intersections had more than one endorhizal morphotype. Quantitative results are reported as a mean of percent abundance ± standard error of the mean.

TABLE 2

Root colonization[a] by morphotypes[b] of endorhizal fungi for flowering plants collected on Truelove Lowland, July 2006.

Genus and species [# plants; # intersections]	AM hypha	FE hypha	FE arb	FE ves	SE hypha	DSE hypha	MS	Intracell coils	Total colonization
Cassiope tetragona [1; 59]	10	35	6	2	38	19	3	5	76
Cerastium sp. [2; 107]	0±0	0±0	0±0	0±0	3.3±3.3	2.0±2.0	0±0	0±0	5.3±1.2
Dryas integrifolia [5; 782]	0.3±0.2	44.8±10.1	5.0±2.7	0±0	55.9±7.9	7.3±2.7	1.5±0.9	1.4±0.9	64.4±5.3
Epilobium latifolium [6; 593]	2.8±1.5	69.1±3.6	9.6±3.2	0±0	44.2±3.6	1.2±1.2	2.1±1.2	1.8±1.1	79.6±2.6
Eriophorum sp. [3; 405]	2.4±2.4	51.2±1.8	2.7±1.6	26.7±11.1	93.3±5.8	0±0	5.2±5.2	5.2±5.2	94.4±2.4
Melandrium sp. [2; 382]	0±0	20.1±7.6	7.3±3.9	1.2±1.2	10.2±1.4	0±0	3.1±2.4	0.5±0.5	30.8±3.6
Oxyria digyna [2; 183]	0±0	94.6±0.3	3.7±1.1	5.1±5.1	96.8±3.2	1.4±1.4	7.7±0.1	7.6±0.4	99.4±0.9
Papaver radicatum [6; 559]	0±0	81.8±6.8	5.6±2.9	1.6±1.2	65.3±6.5	6.2±3.7	2.1±1.6	2.1±1.6	29.9±3.7
Pedicularis spp. [12; 966]	2.4±1.8	50.6±9.1	5.8±5.4	2.3±1.3	67.6±5.0	1.0±0.9	3.0±1.4	3.0±1.4	80.2±5.5
Saxifraga spp. [16; 1886]	1.0±0.5	34.5±7.4	2.9±1.5	0.9±0.3	45.6±7.7	1.1±0.5	1.2±0.6	0.2±0.2	62.7±7.4
All genera [55; 3988]	1.3±0.5	49.6±4.1	5.0±1.4	2.6±1.0	27.8±3.8	2.4±0.7	2.2±0.5	3.8±0.5	71.1±3.6

[a] Endorhizal colonization was determined using the multiple quantitation microintersect method. Percent abundance is given as mean±standard deviation. Up to 200 intersections were assessed per plant, given sufficient root length; for shorter roots, the entire length was assessed; total # of intersection is the sum for the genus. Error is expressed as standard error of the mean.

[b] Fungal morphotypes were arbuscular mycorrhiza hyphae (AM hyphae); fine endophyte hyphae (FE hyphae), and associated arbuscules (FE arb) and vesicles (FE ves); septate endophyte (SE hyphae) and dark septate endophyte (DSE hyphae) hyphae, microsclerotia (MS), and intracellular coils (Intracell coils). SE and DSE were distinguished by whether they could fluoresce (SE) after staining with lactofuchsin.

SOIL BAITING

Soil samples were collected with each plant root system, and stored at −20 °C. Samples that were within 10 cm of each other were considered to be from the same site. To assess whether these soil samples contained viable endorhizal fungal propagules, they were thawed, mixed with sterile vermiculite, placed in 5 cm × 5 cm square pots, planted with wheat (*Triticum aestivum* cv. Conway), and grown in a greenhouse for 10 weeks. At harvest, roots were washed free of soil, and random samples were taken as described by Allen et al. (2006). These were fixed, cleared and stained, and assessed for endorhizal morphotypes as previously described.

Results

Plants in the 10 genera sampled for this study spanned 9 families. Both *Cerastium* and *Melandrium* are in the Caryophyllaceae. Apart from four species of *Saxifraga* and three of *Pedicularis*, genera were represented by single species. *Armeria* roots were not examined using microscopy; however, a soil sample that had contained *Armeria* roots was included in the soil-baiting study. For *Dryas*, which can form ectomycorrhizae that are macroscopically characterized by determinate lateral root branches, only unbranched root segments were selected for this study.

Fungal colonization in root samples was assessed using the MQM method at 30 to 200 intersections that were equally spaced about 400 μm apart; the number of intersections depended on the length of root available. As described by Ormsby et al. (2007) and Walker et al. (2010), CLSM imaging consistently captured more detail than wide-field epifluorescence, particularly for fine endophytes, but the latter had greater depth of focus and proved more practical for quantitation.

Total endorhizal fungal colonization for plants collected from Truelove Lowland in July 2006 ranged from 5% in *Cerastium* to 99% in *Oxyria* (Table 2). AM hyphae were rare, found in <3% of all the intersections examined, and AM vesicles, arbuscules, and hyphal coils were not found. DSE hyphae were infrequent, found only in some *Pedicularis* samples. Overall, 71% of the root intersections contained at least one endorhizal morphotype.

All of the species of Truelove Lowland plants we sampled hosted SE (Fig. 2). The average abundance was 23%, ranging from 3% in *Cerastium* to 97% in *Oxyria* (Table 2). SE were frequently associated with microsclerotia (Fig. 2b), average abundance 13%, and the abundance of these two structures was positively correlated ($r^2 = 0.274$). Septate hyphae that had clamp connections were associated with one *Dryas*, one *Eriophorum*, two *Papaver*, and four *Saxifraga* root systems of the 55 total in the study. The average abundance of hyphae with clamp connections for these eight samples was 13%.

The average abundance for colonization was higher for FE (Fig. 3a) than SE, with hyphae recorded at 50% of root intersections; however, FE hyphae were not found in *Cerastium*. Otherwise, FE colonization ranged from 20% in *Melandrium* to 94% in *Oxyria* (Table 2). In contrast to FE hyphae, the vesicles (Fig. 3b) and arbuscules (Fig. 3c) sometimes associated with FEs at other High Arctic sites (e.g. Allen et al., 2006) were relatively uncommon in these Truelove Lowland samples (Table 2).

Cassiope tetragona, a member of the Ericaceae, contained ericoid mycorrhizae, which are characterized by hyphae growing between as well as within the peripheral cells of the fine lateral roots (Fig. 4) (Peterson et al., 2004). A similar endorhizal morphotype was seen in *Saxifraga* roots (Fig. 5), where hyphae surrounded the root epidermal cells as well as penetrated for intracellular colonization. In *Saxifraga* roots, the intercellular hyphae appeared to be wider than those in *Cassiope* roots (scale bars in Figs. 4 and 5 are each 20 μm). In this study, *Dryas* root segments sampled for imaging and MQM assessment were from regions that had not formed lateral branches characteristic of ectomycorrhizae. These unbranched root segments had a thin sheath of fine hyphae that lacked noticeable septa (Fig. 6a). Hartig nets were not evident, although there was limited intercellular hyphal growth between the epidermal cells (Fig. 6b). The extraradical sheath hyphae were connected to intracellular hyphal coils that filled the outermost layer of the peripheral root cells (Fig. 6c). These intracellular hyphal coils were more tightly packed than those seen with FE arbuscules (Fig. 3c), and were scored separately.
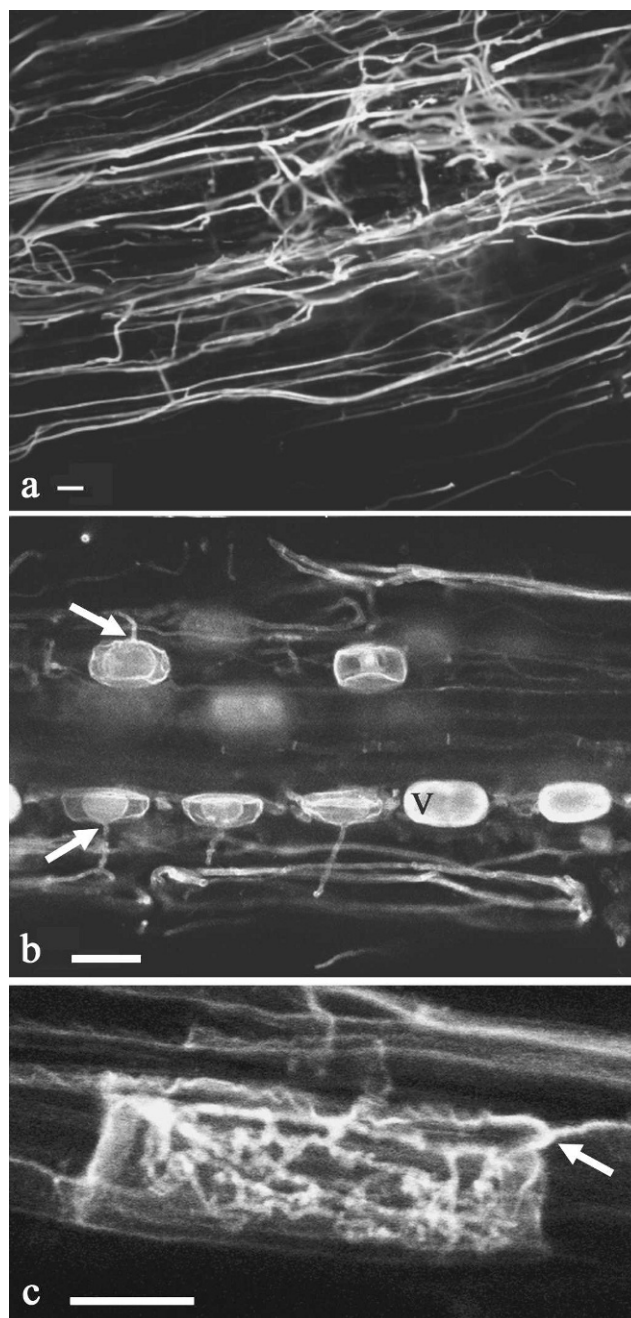
Of the 3988 root intersections assessed for fungal colonization in this study, 8.8% had clear evidence of intracellular colonization: 3.8% with intracellular coils and 5.0% with FE arbuscules (Table 2). Root cell walls are not well contrasted with lactofuchsin fluorescence, so it was not possible to determine whether FE and
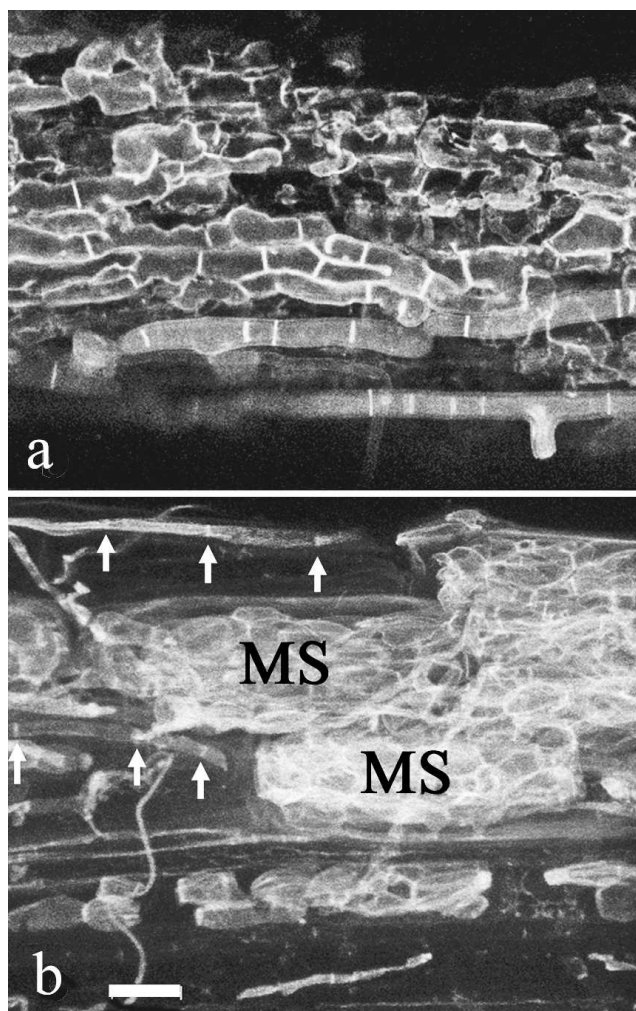
FIGURE 2. Septate endophyte (SE) hyphae that had been stained with lactofuchsin and imaged with confocal laser scanning microscopy. (a) SE on the surface of an *Eriophorum* root, (b) microsclerotia (MS) in an *Eriophorum* root; arrows indicate septa in SE hyphae. Bar in b = 20 μm, for a and b.

FIGURE 3. Fine endophyte (FE) hyphae, vesicles, and arbuscules, stained with lactofuchsin and imaged with confocal laser scanning microscopy. (a) Network of FE hyphae in an *Oxyria digyna* root. *Epilobium latifolium* roots containing (b) FE vesicles (v), and (c) an FE arbuscule. Arrows in b and c indicate junctions between intercellular hyphae and intracellular structures. Bars in a, b, c = 20 μm.

SE hyphal growth was intercellular or intracellular, or both. Intracellular coils were most frequent in *Oxyria* and *Eriophorum*, which also had the most abundant SE colonization (Table 2). FE arbuscules were most frequent in *Epilobium* and *Melandrium,* although FE hyphal colonization was more abundant in *Oxyria* and *Papaver* (Table 2). Intracellular fungal colonization was less frequent in *Dryas* and *Saxifraga* (39 intersections with FE arbuscules and 11 with intracellular coils for *Dryas*; 55 with FE arbuscules and 4 with intracellular coils for *Saxifraga*), although total fungal colonization exceeded 62% for each. Thus, *Dryas* roots that lacked lateral branches characteristic of ectomycorrhizae did host other endorhizal morphotypes.

Soils associated with and collected at the same time as the Truelove Lowland plants, and which had been stored at −20 °C for a year, were assessed for the presence of viable propagules by baiting with wheat. After 10 weeks' growth, MQM analysis of wheat seedling roots showed that SE and FE interactions were predominant (Table 3). The average fungal colonization in the wheat seedlings was 50% for FE hyphae (range 0–100%) and 60% for SE hyphae (range 2–100%), with total colonization averaging 85% (range 34–100%). Thus, soils on Truelove Lowland contain a bank of viable SE and FE fungal propagules.
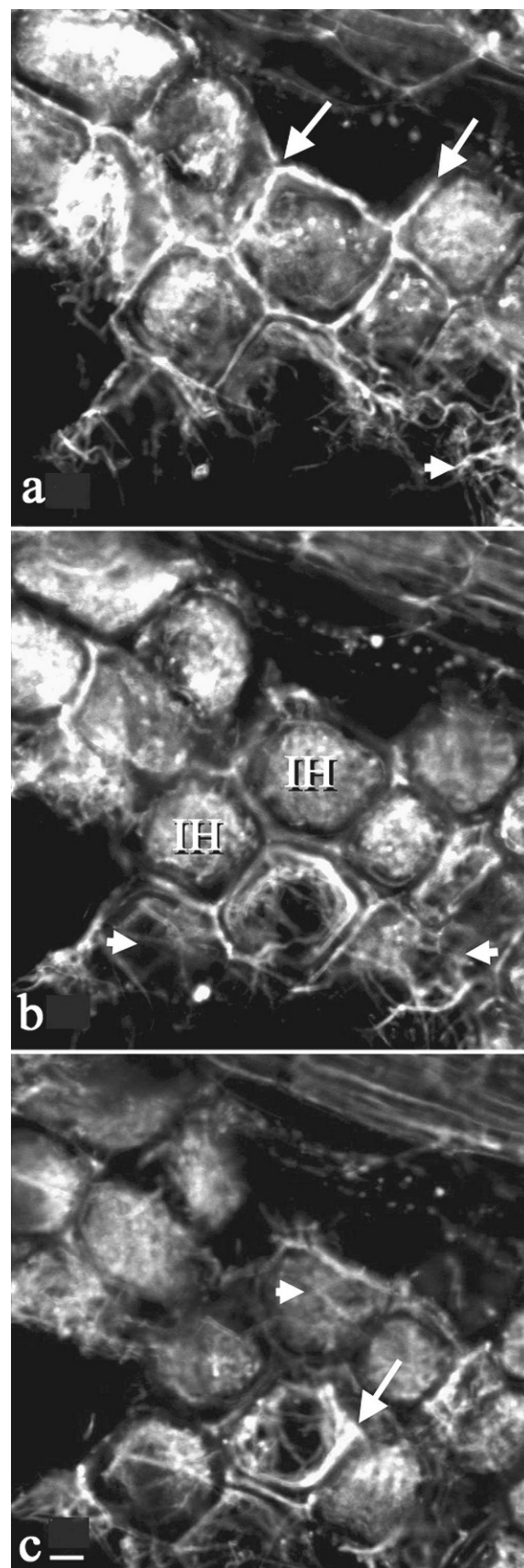
Discussion

We have shown that there is a diversity of endorhizal fungus-root interaction colonization patterns in plants living on the Truelove Lowland tundra. Fungal symbioses characterized by endomycorrhizal interactions had been thought to be rare or absent in High Arctic environments based on the paucity of AM arbuscules detected in plant roots using transmitted light microscopy methods (Bledsoe et al., 1990; Kohn and Stasovski, 1990; Gardes and Dahlberg, 1996). Dalpé and Aiken (1998) and
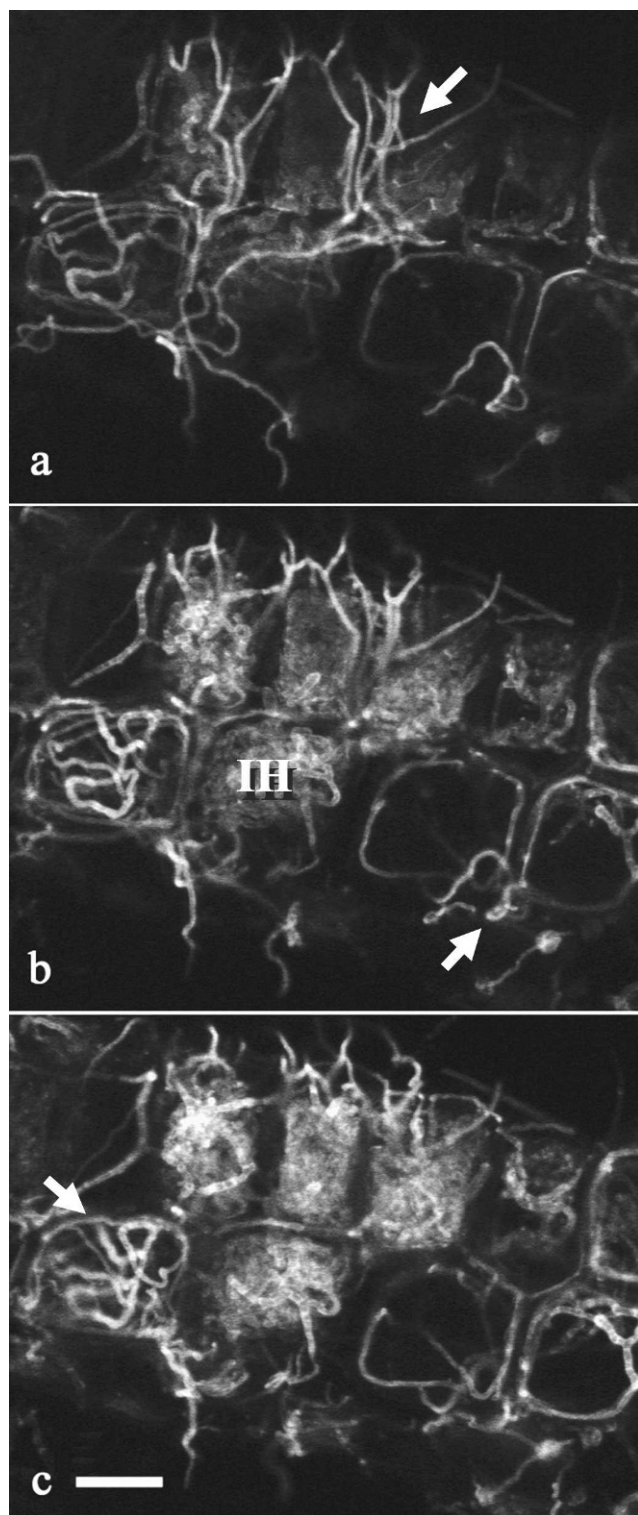
FIGURE 4. *Cassiope tetragona* root stained with lactofuchsin and imaged with confocal laser scanning microscopy, showing ericoid mycorrhizae in the fine roots typical of plants in this family. Images a–c show three focal depths. Intercellular hyphae (arrows) surround root cells that also contain intracellular hyphae (IH). Bar in c = 20 μm, for parts a–c.

FIGURE 5. Endorhizal fungal colonization in a lactofuchsin-stained *Saxifraga hirculus* root imaged with confocal laser scanning microscopy. Images a–c show three focal depths. Hyphae are growing between (arrows) and on the surface (arrowheads) of root cells, which are filled with fine intracellular hyphae (IH). Bar in c = 20 μm, for a–c.

Olsson et al. (2004) showed that AM were present at some High Arctic sites, although in their studies these were uncommon. The development of high-sensitivity imaging and quantification methods (Kaminskyj, 2008) has allowed us to document the
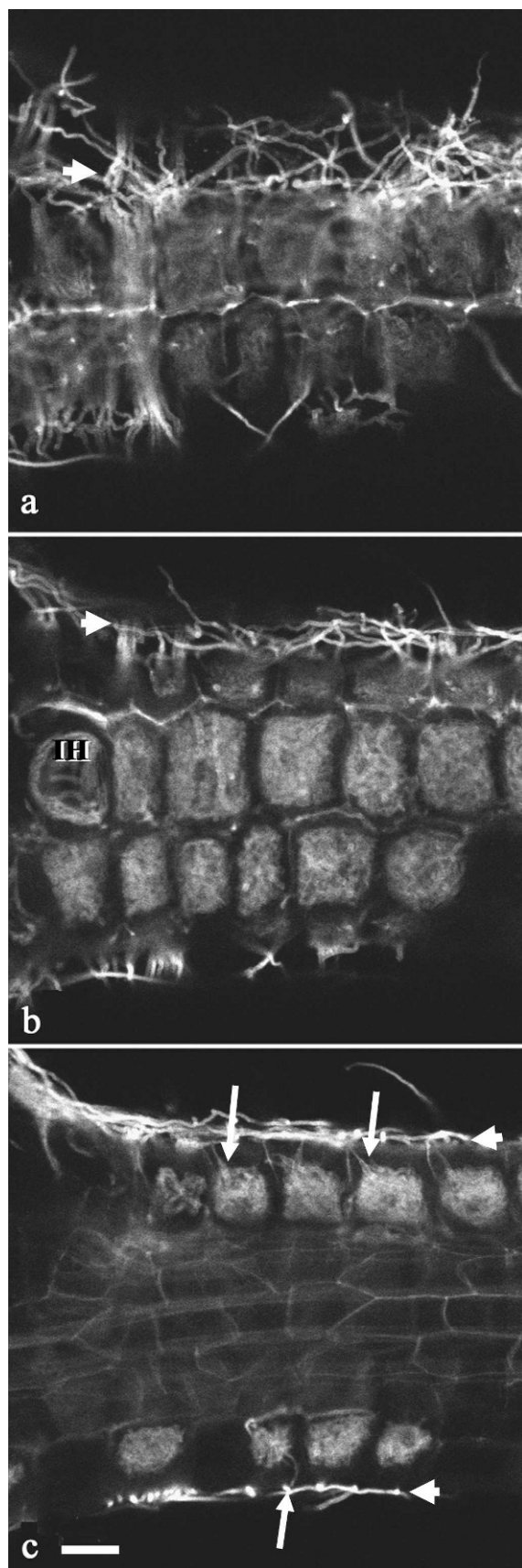
FIGURE 6. Fungal endorhizal colonization in a *Dryas integrifolia* root, from a region of the root that was not associated with the lateral branches characteristic of ectomycorrhizae. This sample had been stained with lactofuchsin and imaged with confocal laser scanning microscopy. Images a–c show three focal depths. (a, b)

diversity and abundance of endorhizal morphotypes in several plant genera collected over the years from many High Arctic and mid-latitude sites (Allen et al., 2006; Ormsby et al., 2007; Hodson et al., 2009; Walker et al., 2010). Thus, we were interested in determining whether comparable associations were truly absent from roots of plants growing at Truelove Lowland, Devon Island, as previous studies seemed to indicate (e.g. Bledsoe et al., 1990). An opportunity arose to obtain specimens collected for this purpose from Truelove Lowland in 2006, allowing us to address this important question.

The disparity between our current findings and those of previous workers, especially Bledsoe et al. (1990), may be largely

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Surface view of a thin sheath of hyphae (arrowhead) that surrounded the root. (b, c) Epidermal cells contain coils of intracellular hyphae (IH) that are attached (arrows) to hyphae of the sheath on the root surface (arrowheads). Bar in c = 20 μm, for a–c.

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attributed to development of a more sensitive imaging method. In particular, we were able to recognize the prevalence of FE, which are difficult to detect using transmitted light microscopy (Thippayarugs et al., 1999; Allen et al., 2006; Walker et al., 2010). We believe that FE are functionally comparable to AM. Both form intracellular arbuscules and vesicles (Allen et al. 2006; Ormsby et al., 2007; Walker et al. 2010), although those of FE are considerably smaller. Gianinnazzi-Pearson et al. (1981) presented convincing ultrastructural and cytochemical evidence that FE are involved in nutrient transfer. In a study of endorhizal fungi in *Ranunculus* roots from sites spanning 52°N–82°N, we found that in contrast to AM, FE were increasingly abundant at higher latitudes, particularly above 66°N (Walker et al., 2010). We suggest that if previous workers could have routinely detected FE hyphae and arbuscules, their interpretation of the rarity of endomycorrhizal fungi in plants growing on high latitude sites would have been different. Furthermore, there has been speculation that endorhizal morphotypes represented by SE might have nutrient acquisition and transfer function (Våre et al., 1992; Jumpponen, 2001; Olsson et al., 2004). Together, the differences between our recent and current results and those previous studies derive from the imaging methods used to detect the fungi, and to the range of endorhizal morphotypes considered.

Ericoid mycorrhizae were readily detected in fine roots of *Cassiope tetragona*, a member of the Ericaceae. These had the expected morphology of fine hyphae ensheathing as well as penetrating the outer layer of the root cortical cells in the fine roots characteristic of this family (Peterson et al., 2004, 2006). Unexpectedly, similar endorhizal morphotypes were also seen in *Saxifraga* (Saxifragaceae) and in unbranched root segments of *Dryas* (Rosaceae), although in both cases the diameter of the hyphae appeared to be narrower than for ericoid mycorrhizae. Synthesized ectomycorrhizae between *Dryas integrifolia* roots and *Hebeloma cylindrosporum* have been shown to form additional root cell layers and lateral branches and are characterized by mantles and Hartig nets (Melville et al., 1987). However, for the *Dryas* samples of the present characterization project we avoided any root segments with lateral branching. Our recently implemented lactofuchsin epifluorescence method is excellent for visualizing Hartig nets, even for samples where they were not anticipated (Fig. 5 *in* Hodson et al., 2009), so we are confident that they would have been detected had they been present. Thus, we conclude that the occurrence of this endorhizal morphotype in *Dryas* roots is novel for this genus.

Peterson et al. (2004) used the term "ectendomycorrhizae" to describe an endorhizal morphotype that produces Hartig nets and also penetrates the cells of some *Pinus* and *Larix* roots; they specified the use of the term "ectendomycorrhizae" exclusively for members of the Pinaceae. An increasing number of angiosperm groups have been found with endorhizal morphotypes that include intercellular and intracellular hyphae. These are: the ericoid and arbutoid mycorrhizae (Ericaceae), cistoid mycorrhizae (Cistaceae, Malvales), orchidoid mycorrhizae (Orchidaceae), and monotropoid mycorrhizae (Monotropaceae) (Peterson et al., 2006). We suggest the general term "ectendiform" to describe a fungal colonization pattern showing both intercellular and intracellular patterns, in preference to creating additional new categories for the endorhizal morphotype such as we have identified in *Dryas* and *Saxifraga*, or to extending current categories between plant families or higher-level taxa. Once the fungal partners are identified using molecular methods, and the nutritional relationships clarified, it may be possible to parse these morphologically similar colonization patterns into functional classes, comparable to the Rodriguez et al. (2009) analysis of fungal endophytes.

Tundra soils have low levels of available phosphorus and nitrogen, as well as low soil organic carbon (Haselwandter et al., 1983; Langley and Hungate, 2003; Read et al., 2003). Symbiotic fungal relationships may be necessary for plants to survive in High Arctic tundra environments, as they are in lower latitudes (Smith and Read, 1997; Rodriguez et al., 2009; Kranabetter and MacKenzie, 2010). Although there is substantial variability in abundance of endorhizal fungi amongst the different plant genera from Truelove Lowland, it is unlikely that this variability could be related to sampling, as the endorhizal fungi we describe in this paper do not produce a macroscopic phenotype, and therefore the plant roots were sampled without bias. To further resolve the basis of this variability, the endorhizal morphotypes would have to be identified using molecular methods, their propagules isolated, and then assessed for colonization abundance of Truelove plants in an experimental setting. This will require additional sampling, which is now justified by our documentation of their abundance.

In order to assess whether fungal propagules were likely to have survived in the soil surrounding the Truelove Lowland plant root systems, we baited soil samples with wheat. We recognize that fungal detection in soil baiting experiments can be affected by the plants chosen (Sýkorová et al., 2007). Bledsoe et al. (1990) had previously assessed Truelove Lowland soil samples for propagules by using *Andropogon* (Poaceae) and *Melilotus* (Fabaceae) seedlings as bait, but found no evidence of endomycorrhizal associations after 8 weeks. A variety of taxa have been used in baiting studies (e.g. Sýkorová et al., 2007). Wheat grows well in greenhouse conditions, and was successfully used by Fester et al. (1999) to generate reproducible and abundant AM root colonization from soil propagules in as little as 5 weeks. We found abundant fungal colonization of wheat seedling roots, 10 weeks after planting seeds in Truelove Lowland soil that had been stored frozen for a year. We suspect that a potentially important experimental difference is that Bledsoe et al. (1990) used soil samples that had been air-dried, whereas our soil samples were stored frozen at −20 °C, then thawed and mixed with sterile vermiculite just before planting.

Further studies to determine the roles played by endorhizal fungi in high latitudes, and molecular analysis to identify the types of fungi present, represent important areas of future research. Nevertheless, MQM analysis does address the importance of these fungi by illustrating their abundance. Our new microscopy techniques have allowed us to see what Bledsoe et al. (1990) and others could not: that endorhizal morphotypes are diverse, widespread, and abundant in plant roots of Truelove Lowland, Devon Island, consistent with our observations from other High Arctic sites (Allen et al., 2006; Ormsby et al., 2007; Hodson et al., 2009; Walker et al., 2010). Because of their abundance, these endorhizal associations are predicted to play significant, and perhaps essential roles in the ecosystems of northern high latitudes, and deserve further examination.

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