

Quantitation of endorhizal fungi in High Arctic tundra ecosystems through space and time: the value of herbarium archives

Alana Ormsby, Emily Hodson, Yang Li, James Basinger, and Susan Kaminskyj

Abstract: Mycorrhizal fungi are widespread in temperate and tropical regions, but generally are thought to be relatively depauperate at high latitudes. The potential impact of global warming on the polar ecosystems has renewed interest in research into tundra soil microbiota. Although logistical impediments limit field access, herbarium accessions are a potential resource for surveying mycorrhizal distribution. We present: (i) a method for examining fungi in roots of herbarium specimens that provides morphological preservation comparable to formalin fixation; and (ii) a multiple quantitation method to assess diverse morphotypes. Arbuscular mycorrhizae, fine endophytes, and septate endophytes were widespread in Asteraceae roots from Axel Heiberg and Ellesmere islands, Arctic Canada, during 2004. Roots from the same species collected from this region since 1982, stored in our herbarium, consistently contained abundant endorhizal fungi. Although 2004 was one of the coolest growing seasons in the survey, mycorrhizal abundance was highest in that year. Endorhizal fungi are likely to be important for plant survival and soil-forming processes in High Arctic tundra environments, and may be sensitive to climate variation.

Key words: High Arctic, arbuscular mycorrhizal fungi, fine endophyte fungi, septate endophyte fungi, confocal epifluorescence microscopy, multiple quantitation method.

Résumé : Les champignons mycorrhiziens sont répandus dans les régions tempérées et tropicales, mais on croit généralement qu'ils sont relativement moins abondants sous les hautes latitudes. L'impact potentiel du réchauffement global sur les écosystèmes polaires renouvelle l'intérêt pour la recherche sur les microorganismes édaphiques de la toundra. Alors que des empêchements logistiques limitent l'accès au terrain, les spécimens d'herbiers constituent une ressource potentielle pour observer la distribution des mycorrhizes. Les auteurs présentent : (i) une méthode pour examiner les champignons dans les racines de spécimens d'herbiers, qui présentent une conservation morphologique comparable à la fixation au formol; (ii) une méthode de quantification multiple pour évaluer divers morphotypes. Les mycorrhizes arbusculaires, les endophytes fins, et les endophytes septés, étaient largement répandus chez les racines des Asteraceae provenant des îles d'Axel Heiberg et Ellesmere, de l'Arctique canadien, en 2004. Les racines de ces mêmes espèces récoltées dans la même région depuis 1982, conservées dans les herbiers des auteurs, contiennent régulièrement des champignons endorhiziens. Bien que 2004 fut une des saisons de croissance les plus froides pour la période observée, l'abondance des mycorrhizes a été la plus grande cette année là. Les champignons endorhiziens sont vraisemblablement importants pour la survie des plantes et la pédogénèse dans les environnements de tundra du Haut Arctique, et ils pourraient être sensibles aux variations climatiques.

Mots-clés : Haut Arctique, champignons mycorrhiziens arbusculaires, champignons endophytes fins, champignons endophytes septés, microscopie confocale en épifluorescence, méthode de quantification multiple.

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Introduction

The importance of mycorrhizal fungi is well documented for temperate and tropical plant communities (Smith and Read 1997) and has been suggested to have been a prerequisite for land colonization (Malloch et al. 2000). There are

relatively few studies of endomycorrhizae from Arctic tundra environments (reviewed in Allen et al. 2006), in part because of logistical difficulties. However, since the earliest explorations of the region, plants have been collected from diverse Arctic sites and preserved as herbarium specimens. These represent a potentially untapped resource for exploring the presence and diversity of endorhizal fungi in Arctic environments.

Endophytic fungus – plant root interactions in herbaceous plants include arbuscular mycorrhizae (AM), fine endophytes (FEs), and septate endophytes (SEs) (Peterson et al. 2004). Percent root colonization is widely used as an index for assessing the relative significance of an endorhizal interaction. The microscopic grid intersect method (McGonigle et al. 1990), for example, provides reliable results for situations such as pot-culture assays. However, unlike cultivated

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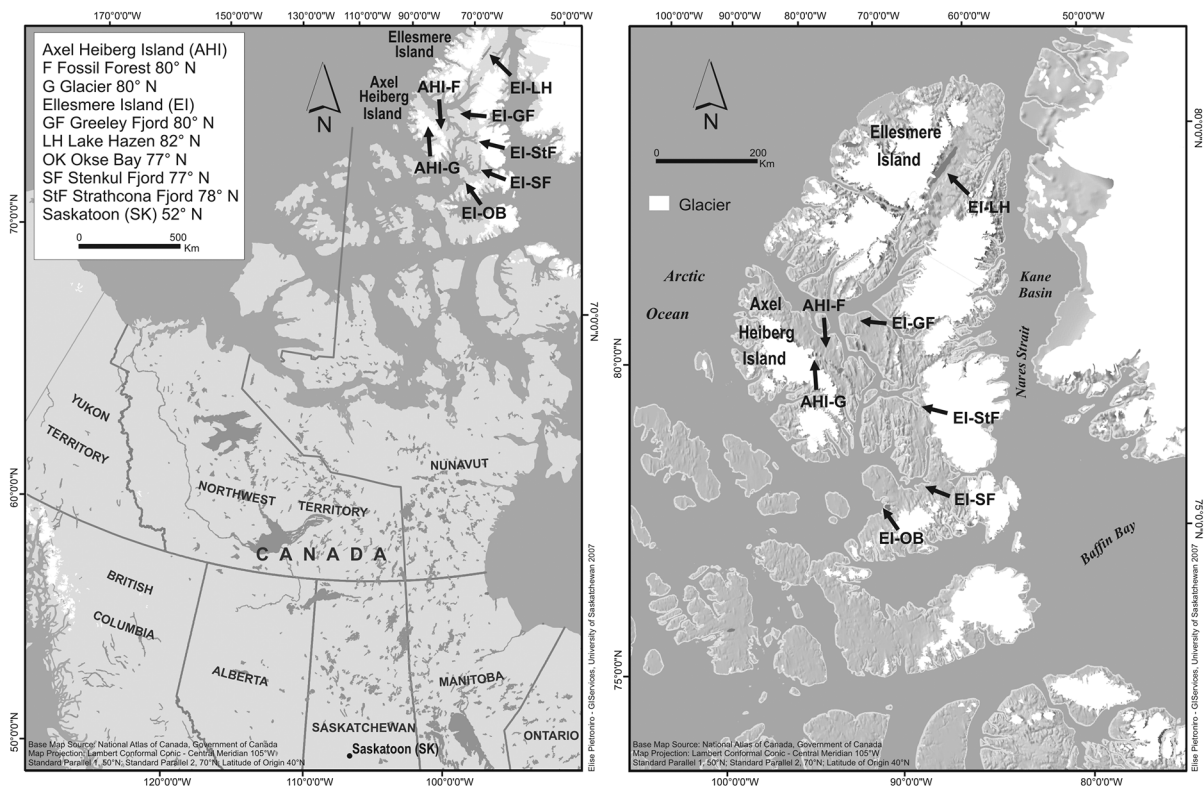
A. Ormsby and J. Basinger. Department of Geological Sciences, University of Saskatchewan, 114 Science Place, Saskatoon SK S7N 5E2, Canada.

E. Hodson, Y. Li, and S. Kaminskyj.¹ Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon SK S7N 5E2, Canada.

¹Corresponding author (e-mail: Susan.Kaminskyj@usask.ca).

Table 1. Collection sites for specimens in this study.

Location	Latitude	Longitude	Year	Species	Habitat
Axel Heiberg Island, fossil forest	79°55'N	89°02'W	1985	<i>Erigeron compositus</i>	Clay soil on slope
			1988	<i>Taraxacum phymatocarpum</i>	Moist bench
			1990	<i>Erigeron compositus</i>	Moist, silty mud
			1990	<i>Erigeron eriocephalus</i>	Dry gravelly hillside
			1991	<i>Arnica alpina</i>	Dry rocky tundra
Axel Heiberg Island, glacier camp	79°53'N	89°33'W	2004	<i>Erigeron compositus</i>	Dry rocky tundra
			2004	<i>Erigeron eriocephalus</i>	Dry rocky tundra
			2004	<i>Taraxacum hyparcticum</i>	Vegetative bank
			2004	<i>Taraxacum phymatocarpum</i>	Vegetative bank
Ellesmere Island, Lake Hazen	82°03'N	69°10'W	1988	<i>Erigeron eriocephalus</i>	Moist hill
			1988	<i>Taraxacum hyparcticum</i>	Moist moss meadow
Ellesmere Island, Greeley Fjord	80°16'N	85°75'W	1985	<i>Taraxacum hyparcticum</i>	Moist meadow
Ellesmere Island, Fosheim Peninsula	79°44'N	85°35'W	1982	<i>Arnica alpina</i>	Dry sandy slope
			1990	<i>Arnica alpina</i>	Turf
			1993	<i>Erigeron eriocephalus</i>	Moist hill
Ellesmere Island, Okse Bay	77°07'N	86°42'W	1988	<i>Taraxacum phymatocarpum</i>	Moist bench

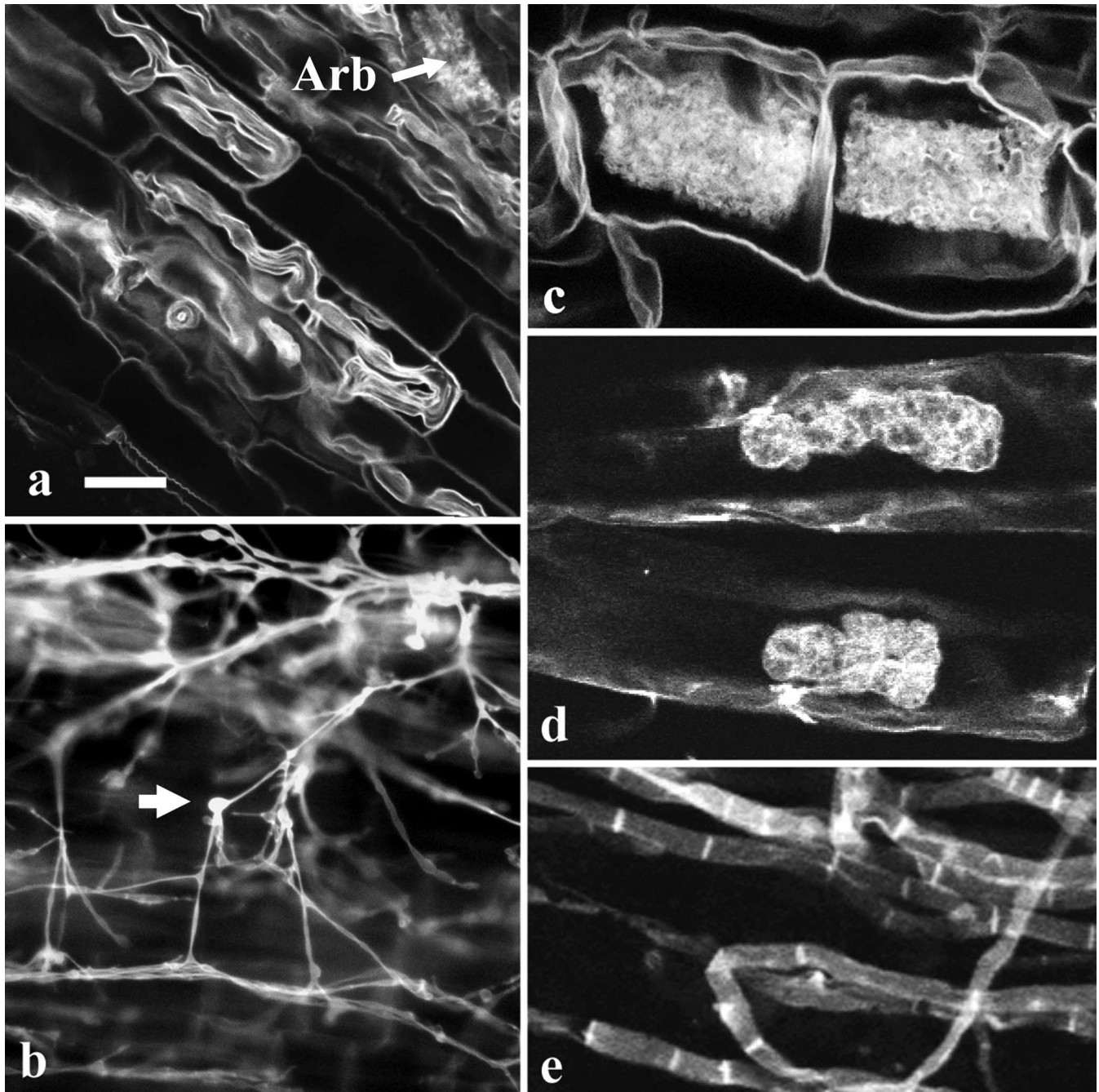
Fig. 1. Map and satellite images of the field sites considered in this study. Details are given in Table 1.

soils, plant communities growing in undisturbed soils typically contain multiple types of endorhizal fungi. Molecular identification using fungal DNA from soils or roots (e.g., Ma et al. 2005) does not address abundance, and sequence amplification may be limited by primer specificity, so there remains an important role for direct quantitation. A robust direct quantitation method should be inexpensive, reproducible, and should capture information on colonization by multiple endorhizal morphotypes.

To determine if dried herbarium material preserves an ad-

equate record of endorhizal fungi, we used the lactofuchsin fluorescence technique described in Allen et al. (2006) to examine endorhizal fungi in roots of herbarium specimens. To assess endorhizal diversity, we developed a multiple quantitation method (MQM) that, in its simplest form, is equivalent to the McGonigle et al. (1990) procedure. Here, we used MQM to quantify colonization of roots of three genera in the Asteraceae collected between 1982 and 1993 from seven sites on Axel Heiberg Island and Ellesmere Island, Canadian Arctic Archipelago, and compared these re-

Fig. 2. Endorhizal fungi in lactofuchsin-stained herbarium specimen roots viewed with confocal epifluorescence (*a, c–e*) and wide-field epifluorescence (*b*) microscopy. (*a*) Arbuscular mycorrhiza (AM) in *Taraxacum phymatocarpum*, showing hyphal coils, and arbuscules (Arb). (*b*) Fine endophyte (FE) hyphae in *Taraxacum hyarcticum*, with small vesicles (arrow). (*c*) AM arbuscules in *Erigeron eriocephalus*, (*d*) FE arbuscules in *Erigeron compositus*, (*e*) septate endohytes in *E. eriocephalus*. Scale bar (*a–e*) = 20 μm .



sults with formalin-fixed and herbarium material from the 2004 field season.

Materials and methods

Plant materials

Samples collected from the Canadian High Arctic are described in Table 1. Slides of *Taraxacum hyarcticum* Dahlst., *T. phymatocarpum* J. Vahl., *Erigeron compositus*

Pursh., and *E. eriocephalus* J. Vahl. containing lactofuchsin-stained roots used in Allen et al. (2006) had been archived, so where possible these were reanalyzed as described below. Root samples of *Arnica alpina* L., preserved in formalin in 2004 and stored at 4 °C, were prepared to replace glycerol-mounted slides that were no longer useable.

Specimens of Asteraceae collected on Axel Heiberg Island and Ellesmere Island by Basinger and colleagues since 1982 are curated in the W.P. Fraser Herbarium, University

Table 2. Abundance of endorhizal fungi^a at sites^b on Axel Heiberg Island and Ellesmere Island in the Canadian High Arctic.

(A) Arbuscular mycorrhiza structures.								
Date	Species	Site ^b	AMA	AMV	AMH-L	AMH-M	AMH-H	AMH-tot
1982	<i>Arnica alpina</i> (2)	EI-FP	1.5±1.5	0±0	0±0	0±0	27.0±27.0	27.0±27.0
1985	<i>Erigeron compositus</i>	EI-GF	0	0	0	0	0	0
	<i>Erigeron compositus</i>	AHI-F	0	0	0	0	0	0
	<i>Taraxacum phymatocarpum</i>	EI-GF	0	3	7	3	17	27
1986	<i>Arnica alpina</i>	EI-FP	0	0	0	5	7	12
	<i>Erigeron eriocephalus</i> (2)	EI-LH	1.5±1.5	0±0	1.5±1.5	9±6	10±2	19.5±6.5
	<i>Taraxacum phymatocarpum</i> (2)	EI-OB	0±0	0±0	0±0	9.5±2.5	14.5±5.5	24±3
	<i>Taraxacum phymatocarpum</i>	EI-LH	61	0	3	12	70	85
1990	<i>Arnica alpina</i>	EI-FP	0	0	0	2	6	8
	<i>Erigeron compositus</i>	AHI-F	0	0	3	4	4	11
	<i>Erigeron eriocephalus</i> (4)	AHI-F	12.2±5.6	0±0	0.2±0.2	3.2±1.5	47.0±14.8	50.5±15.5
1991	<i>Arnica alpina</i>	AHI-F	0	0	0	2	10	12
1993	<i>Erigeron eriocephalus</i>	EI-SF	0	3	0	18	25	43
2004	<i>Arnica alpina</i> (5)	AHI-G-f	0.8±0.8	2.6±2.6	0±0	0.4±0.4	0.4±0.4	3.0±3.0
	<i>Erigeron compositus</i> (2)	AHI-G	0±0	0±0	0±0	0±0	3±3	3±3
	<i>Erigeron eriocephalus</i> (2)	AHI-G	2.0±2.0	1.0±1.0	0±0	4.0±1.0	17.0±6.0	21.0±5.0
	<i>Erigeron eriocephalus</i>	AHI-G-f	19	0	0	22	30	52
	<i>Taraxacum hyparcticum</i> (5)	AHI-G	15.6±5.7	5.2±1.4	0.6±0.6	84±3.8	20.6±5.6	27.6±6.6
	<i>Taraxacum hyparcticum</i>	AHI-G-f	17	28	3	20	12	35
	<i>Taraxacum phymatocarpum</i> (6)	AHI-G	15.5±3.7	22.0±7.8	1.5±0.9	7.5±3.1	57.8±13.5	65.2±13.4
	<i>Taraxacum phymatocarpum</i> (3)	AHI-G-f	14.7±6.8	8.0±6.0	2.0±1.2	5.0±1.2	34.3±20.8	41.3±22.9
Abundance of types of endorhizal fungi, as pooled for each plant species from all years and all sites								
	<i>Arnica alpina</i> (10)		0.7±0.5	1.3±1.3	0±0	1.1±0.5	7.9±5.2	10.1±5.2
	<i>Erigeron compositus</i> (5)		0±0	0±0	0.6±0.6	0.8±0.8	2.0±1.3	3.4±2.2
	<i>Erigeron eriocephalus</i> (8)		7.5±3.0	0.5±0.3	0.4±0.3	7.9±2.4	29.7±7.5	37.8±7.6
	<i>Taraxacum hyparcticum</i> (6)		15.8±4.6	9.0±4.0	1.0±0.6	10.3±3.7	19.2±4.8	29.8±5.5
	<i>Taraxacum phymatocarpum</i> (13)		13.5±4.3	11.4±3.5	1.8±0.6	6.7±1.1	39.8±7.6	48.1±7.7
Abundance in all species from all years and all sites								
			8.1±1.8	5.2±1.4	0.8±0.2	5.6±0.9	25.5±3.7	29.5±4.1
(B) Fine endophyte and septate endophyte structures, and total colonization								
Date	Species	Site ^b	FEA	FEV	FEH	SEH	Total Col	
1982	<i>Arnica alpina</i> (2)	EI-FP	0±0	0±0	3.5±3.5	67.0±15.0	88.0±6.0	
1985	<i>Erigeron compositus</i>	EI-GF	0	0	36	78	78	
	<i>Erigeron compositus</i>	AHI-F	0	0	0	0	0	
	<i>Taraxacum phymatocarpum</i>	EI-GF	0	0	0	97	100	
1986	<i>Arnica alpina</i>	EI-FP	0	0	0	32	40	
1988	<i>Erigeron eriocephalus</i> (2)	EI-LH	0±0	0±0	0±0	44.5±0.5	51.5±1.5	
	<i>Taraxacum phymatocarpum</i> (2)	EI-OB	0±0	0±0	0±0	0±0	24.0±3.0	
	<i>Taraxacum phymatocarpum</i>	EI-LH	0	3	6	18	97	
1990	<i>Arnica alpina</i>	EI-FP	0	2	16	82	98	

Table 2 (concluded).

(B) Fine endophyte and septate endophyte structures, and total colonization							
Date	Species	Site ^b	FEA	FEV	FEH	SEH	Total Col
1991	<i>Erigeron compositus</i>	AHI-F	6	1	17	35	49
	<i>Erigeron eriocephalus</i> (4)	AHI-F	0±0	0±0	5.5±4.8	67.5±6.0	93.2±5.8
1993	<i>Arnica alpina</i>	EI-FP	0	2	35	12	58
2004	<i>Erigeron eriocephalus</i>	EI-SF	0	0	1	32	62
	<i>Arnica alpina</i> (5)	AHI-G-f	55.6±8.3	20.0±3.7	89.4±5.7	2.8±1.0	91.0±4.8
	<i>Erigeron compositus</i> (2)	AHI-G	13.0±7.0	11.0±5.0	84.0±16.0	12.5±12.5	84.0±16.0
	<i>Erigeron eriocephalus</i> (2)	AHI-G	1±1	1±1	30.5±7.5	52.5±8.5	80.0±80.0
	<i>Erigeron eriocephalus</i>	AHI-G-f	4	0	18	11	63
	<i>Taraxacum hyparcticum</i> (5)	AHI-G	22.2±4.9	30.0±3.3	72.2±11.3	17.2±9.8	86.0±5.7
	<i>Taraxacum hyparcticum</i>	AHI-G-f	15	20	100	55	100
	<i>Taraxacum phymatocarpum</i> (6)	AHI-G	0±0	0±0	1.8±0.6	50.8±14.7	84.8±7.9
	<i>Taraxacum phymatocarpum</i> (3)	AHI-G-f	25.3±10.7	11.3±3.8	60.3±26.8	37.3±9.0	95.7±3.4
Abundance of types of endorhizal fungi, as pooled for each plant species from all years and all sites							
	<i>Arnica alpina</i> (10)		27.8±10.1	10.4±3.6	50.5±13.6	27.4±10.5	82.7±6.3
	<i>Erigeron compositus</i> (5)		6.4±3.6	4.6±3.0	44.2±17.9	27.6±14.4	59.0±16.9
	<i>Erigeron eriocephalus</i> (8)		0.6±0.4	0.2±0.2	10.2±4.3	50.7±6.4	76.1±6.0
	<i>Taraxacum hyparcticum</i> (6)		21.0±4.2	28.3±3.2	76.8±10.4	26.5±10.2	88.3±5.2
	<i>Taraxacum phymatocarpum</i> (13)		5.6±3.4	2.8±1.4	16.5±8.4	42.1±8.6	78.3±7.3
Abundance in all species from all years and all sites							
			11.6±2.9	7.5±1.7	33.8±5.7	36.7±4.4	80.0±3.6

Note: ^aPercentage colonization using the multiple quantitation method (MQM, see Methods). Arbuscular mycorrhiza (AM) structures: AMA, arbuscules; AMV, vesicles; AMH, hyphae. Fine endophyte (FE) structures: FEA, arbuscules; FEV, vesicles; FEH, hyphae; SEH, septate endophyte hyphae. AMH were subdivided by the number of hyphae per intersection: AMH-L, low abundance (1); AMH-M, medium abundance (2–5); AMH, high abundance (>6). Total AMH is the sum of low, medium, and high abundance intersections. Total Col is the percentage of intersections with at least one type of fungal structure. Replicate root systems are reported sequentially. Dark septate endophytes were <10% of SEH. ^bSites: AHI-F, Axel Heiberg Island Fossil Forest (Geodetic Hills); AHI-G, Axel Heiberg Island Glacier; EI-FP, Ellesmere Island Fosheim Peninsula; EI-GF, Ellesmere Island Greeley Fjord; EI-LH, Ellesmere Island Lake Hazen; EI-OB, Ellesmere Island Okse Bay; EI-SF, Ellesmere Island Stenkul Fjord. AHI-G-f samples were from formalin-fixed roots collected at the same time as the herbarium specimens. All data prior to 2004 were from herbarium samples.

of Saskatchewan. Samples were taken from specimens with abundant roots. Depending on the size of the root system, 6–20 cm of lateral root per specimen was removed, representing 10%–25% of the estimated total root length.

Microscopy preparation

Formalin-fixed samples and herbarium samples were treated similarly regarding clearing and staining. The herbarium specimens were slightly more fragile than formalin-fixed roots. Herbarium root samples were rehydrated in room-temperature 10% KOH. Herbarium and formalin-fixed samples were cleared by autoclaving in 10% KOH for 20 min, which then was removed with two washes in room-temperature 70% ethanol. Cleared roots were stained for 3 h at 68 °C in 0.05% acid fuchsin in 85% lactic acid, then destained in two changes of 1:1:1 distilled water – 85% lactic acid – glycerol at 47 °C. This method was adapted from Merryweather and Fitter (1991) and Brundrett et al. (1996) as reviewed in Allen et al. (2006). Other staining methods are reviewed in Verheilig et al. (2005).

Stained roots were mounted in polyvinyl alcohol glycerol (PVAG) medium, adapted from the method of Cunningham (1972). PVAG medium was made by dissolving 5 g polyvinyl alcohol powder (JT Baker, VWR International, Edmonton, Alta.) in 50 mL distilled water at 60 °C with constant stirring (4 h to overnight), to which was added 20 mL glycerol and 0.01% sodium azide. This solution was stored at room temperature. After the specimens were mounted, the PVAG medium was polymerized overnight at 40 °C, and the slide edges were sealed with nail polish. Compared with glycerol-mounted specimens (Allen et al. 2006), the PVAG slides were more robust and easier to clean of immersion oil. PVAG is nonfluorescent in 514 or 543 nm light.

Fungal endophytes were imaged using a Zeiss META 510 confocal laser scanning microscope (www.zeiss.com) equipped with 25× Plan NeoFluar N. A. 0.8 and 63× C-Apochromat N. A. 1.2 multi-immersion objectives, each with differential interference contrast optics. Imaging used 543 nm excitation, 9.9% intensity of a 25 mW beam from a HeNe laser, a HFT 488/543 beam splitter, and a 604–657 nm emission filter. Fluorescence and transmitted light images were collected simultaneously. Additional images and endorhizal quantitation used a Zeiss Axioplan microscope equipped with a 20× N. A. 0.5 Plan Neofluar, a 40× N. A. 0.75 Plan Neofluar, and a 63× N. A. 1.4 Plan Apochromat oil immersion objective, each with differential interference contrast optics. Axioplan wide-field epifluorescence imaging of lactofuchsin-stained material used a BP546 excitation filter, FT580 dichroic mirror, and LP590 emission filter. Images were captured using a Sensys CCD (www.roper.com) driven by MetaVue software (www.image1.com).

Multiple quantitation method

Colonization by fungal root endophytes was assessed using a multiple quantitation method modified from McGonigle et al. (1990). Intersections were taken 1–2 mm apart, as recommended by Brundrett et al. (1996), and examined using 200×, 400×, or 630× total magnification. McGonigle et al. (1990) indicated that intersections should be evenly spaced within a sample, but needn't be the same for all sam-

ples. Roots were examined using wide-field and (or) confocal epifluorescence microscopy. Each intersection was assessed individually for several types of fungal endophyte. These were as follows: 4–6 µm wide aseptate hyphae characteristic of AM; arbuscules and vesicles associated with AM hyphae; 1–1.5 µm wide aseptate hyphae characteristic of FEs; arbuscules and vesicles associated with FE hyphae; and SE hyphae. Intersections not associated with any fungi were scored separately to provide an estimate of total percent colonization. AM hyphae varied considerably in abundance between intersections, so low (1), medium (2–5), and high (>6) abundance AM hyphae were scored separately. Intracellular hyphal coils were not scored separately in this survey; however, coils did not account for many of the high-abundance intersections.

Results are reported for the root systems of individual plants and are expressed as mean ± standard error. Statistical analysis used Statview 1.01 to compare colonization abundance by single factor ANOVA followed by Fisher PLSD.

Results

The collection sites for the specimens used in this study are shown in Fig. 1, and additional locality details are given in Table 1.

Formalin-fixed and herbarium materials were examined following lactofuchsin staining using wide-field epifluorescence and confocal epifluorescence microscopy. Where possible, samples were included from the Allen et al. (2006) study. Confocal imaging consistently captured more detail than wide-field epifluorescence, but the latter had greater depth of focus and was easier to use for quantitation. Formalin-fixed and herbarium specimens provided similar visualization quality for endorhizal fungi, although formalin-fixed specimens typically had more finely detailed arbuscules. Herbarium material contained AM intracellular coils (Fig. 2a), arbuscules (Figs. 2a and 2c), and vesicles (not shown), FE hyphae and vesicles (Fig. 2b) and arbuscules (Fig. 2d), and septate endophytes (Fig. 2e). Comparison of endorhizal abundances from formalin-fixed and herbarium material collected in 2004 showed their abundance was statistically similar for each type of structure. This excellent preservation in herbarium root material enabled us to survey endorhizal fungi associated with Asteraceae between 1982 and 1993 at seven sites on Axel Heiberg Island and Ellesmere Island spanning 77–82°N.

Quantitation of endorhizal fungi using MQM is shown in Table 2. Plants collected at the same time and site varied in fungal colonization. The results in Table 2 are based on individual root systems, each assessed at 50–100 intersections. The percent colonization rates obtained with MQM for formalin-fixed roots were consistent with those presented by Allen et al. (2006), with the advantage of distinguishing between types of arbuscules and vesicles, and of hyphae. We also assessed different intensities of AM hyphal colonization, since this varied widely. Typically, where present, AM hyphae were abundant regardless of whether they were associated with arbuscules or vesicles. Only some of the AM hyphal abundance was due to intracellular hyphal coils.

For *E. eriocephalus* and *T. phymatocarpum* collected in 2004, where we had both herbarium specimens with abun-

dant roots and formalin-fixed samples, the endorhizal abundance between specimens preserved by drying or fixation was statistically similar ($P > 0.05$, ANOVA).

Fungal endorhizal colonization varied widely between samples from different species, sites, years, and types of endorhizal fungus (Table 2). However, for the 44 plants for which we had data, only 3/44 had less than 30% total colonization, and 30/44 had at least 70% total colonization, with AM, FE, and SE contributing approximately equally. Colonization was higher for 2004 than for any other year ($P < 0.001$, ANOVA), and was higher for the Axel Heiberg Glacier site than elsewhere ($P < 0.003$, ANOVA). The Glacier site (80°N) is about midway in our latitudinal transect (77°N to 82°N), and at a similar latitude to the Greely Fjord, Fosheim Peninsula and Fossil Forest sites, but unlike those sites, it is very close to a glacier and in a microenvironment that appeared harsher than any other site in this survey. There were no major differences attributable to soil type, and all the specimens would have been growing in the 15–20 cm thick active layer overlying the permafrost.

Discussion

Studies of endorhizal fungal abundance for plants growing in extreme climates such as in the Canadian High Arctic Archipelago are constrained by short growing seasons and by challenging logistics. Furthermore, given the expectation from the literature that AM at these sites are depauperate (reviewed in Allen et al. 2006), it has been understandably difficult to undertake fieldwork. We have shown here that it is practical to use roots from herbarium collections to broadly survey endorhizal distributions, and to identify promising sites for future in-depth investigation. For example, this is the first study to show that multiple types of endorhizal fungi are widely distributed in Asteraceae collected across a latitudinal gradient in the Canadian High Arctic, and that these have been present at these sites for more than two decades. Techniques are available for extracting DNA from herbarium specimens, which in future will permit parallel molecular and morphological analyses.

The MQM data presented herein are considerably more informative than the quantitative data of Allen et al. (2006). MQM shows that AM, FEs, and SEs are present in plants growing in High Arctic soils, consistent with findings of Olsson et al. (2004) and Dalpé and Aiken (1998). Gianinazzi-Pearson et al. (1981) provided ultrastructural evidence for nutrient exchange at FE arbuscules, and Jumpponen (2001) has suggested that septate endophytes might have a mycorrhizal role. Most root systems examined in this study hosted all three types of endorhizal interaction. Allen et al. (2006) showed that FEs could produce arbuscules in roots of High Arctic plants, and MQM shows that FE arbuscules were often as, or more abundant, than AM arbuscules. Thus, both FE and SE fungi have the potential to play important roles in soil microbiota – plant root interactions.

Endorhizal fungi varied in abundance in different years. Abundance was not simply correlated with the weather of the particular year, since 2004 was one of the coolest summers of all the years for which we have samples, and yet had the highest total colonization rates. Although we have more samples for 2004, and overall they had signifi-

cantly more endorhizal fungi, there is no reason to believe that this was a unique event. Indeed, since AM and FE are obligate biotrophs whose propagules are soilborne, finding AM and FE indicates there must have been viable propagules from previous years. Amazingly, this was even so for plants collected from dry gravelly hillsides and rocky tundra sites, where it is difficult to imagine isolated individuals being able to survive. SE fungi have Ascomycete affinities (Jumpponen 2001) and thus may form spores with higher dispersal potential. SE abundance was more consistent between years and sites than were AM or FE.

In summary, we have shown that the roots of herbarium specimens can provide valuable material for studying endorhizal fungi. The MQM method described herein provided detailed information about the types and relative abundance of endorhizal fungi, particularly when used in conjunction with lactofuchsin-stained material viewed with epifluorescence microscopy. PVAG mounting medium is useful for fluorescence studies and is superior to glycerol. Most importantly, we have shown that endorhizal fungi including AM, FE, and SE are widespread and relatively abundant in roots of Asteraceae plants collected from many sites over many years in the Canadian High Arctic, and archived as herbarium mounts. Future studies to elucidate roles played by endorhizal fungi in the High Arctic have the potential to increase our understanding of the relation between the soil fungal microbiota, plant survival, and soil-forming processes. The archive of material that exists in herbaria furthermore represents an untapped resource for spatial and temporal studies of endorhizal abundance that can be correlated with climatic parameters and climate change.

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