Functional Ecology

BRITISH ECOLOGICAL SOCIETY

Functional Ecology 2017, **31**, 116–126 doi: 10.1111/1365-2435.12695

Host phenology and potential saprotrophism of ectomycorrhizal fungi in the boreal forest

Stefan F. Hupperts*[,1], Justine Karst[1], Karin Pritsch[2] and Simon M. Landhäusser[1]

[1]*Department of Renewable Resources, University of Alberta, Edmonton, Alberta T6G 2H1, Canada; and* [2]*Helmholtz Zentrum München GmbH, German Research Center for Environmental Health, Institute of Biochemical Plant Pathology, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany*

Summary

1. Phenology-induced changes in carbon assimilation by trees may affect carbon stored in fine roots and as a consequence, alter carbon allocated to ectomycorrhizal fungi. Two competing models exist to explain carbon mobilization by ectomycorrhizal fungi. Under the 'saprotrophy model', decreased allocation of carbon may induce saprotrophic behaviour in ectomycorrhizal fungi, resulting in the decomposition of organic matter to mobilize carbon. Alternatively, under the 'nutrient acquisition model', decomposition may instead be driven by the acquisition of nutrients locked within soil organic matter compounds, with carbon mobilization a secondary process.

2. We tested whether phenology-induced shifts in carbon reserves of fine roots of aspen (*Populus tremuloides*) affect potential activity of four carbon-compound degrading enzymes, β-glucuronidase, β-glucosidase, *N*-acetylglucosaminidase and laccase, by ectomycorrhizal fungi. Ectomycorrhizal roots from mature aspen were collected across eight stands in north-eastern Alberta, Canada, and analysed during tree dormancy, leaf flush, full leaf expansion and leaf abscission. We predicted potential extracellular enzyme activity to be highest when root carbon reserves were lowest, should host phenology induce saprotrophism. Further, we anticipated enzyme activity to be mediated by invertase, a plant-derived enzyme which makes carbon available to fungal symbionts in the plant–fungus interface.

3. Root carbon reserves were positively correlated with invertase, suggesting phenology may affect carbon allocation to ectomycorrhizal fungi. However, of the four enzymes, host phenology had the largest effect on β-glucuronidase, but activity of this enzyme was not correlated with root carbon reserves or invertase. Low-biomass ectomycorrhizas had greater potential laccase activity than high-biomass ectomycorrhizas, highlighting discrete functional traits in fungi for litter decomposition.

4. Our results suggest that the decomposition of organic matter may be driven by foraging by fungi for nutrients locked within organic compounds rather than for mobilizing carbon. Furthermore, the potential ability to degrade lignin was more common in low-biomass ectomycorrhizas when compared to high-biomass ectomycorrhizas.

Key-words: decomposition, ectomycorrhizal exploration type, extracellular enzymes, invertase, nonstructural carbohydrates, nutrient cycling, phenology, *Populus tremuloides*

Introduction

Ectomycorrhizal fungi form a symbiotic relationship with trees, functionally extending the roots of their host tree and providing mineral nutrients in exchange for photosynthetically derived carbon. This symbiosis is particularly important in boreal forests, where slow decomposition rates drive nutrient limitation (Read, Leake & Perez-Moreno 2004). Moreover, trees in the boreal forest undergo dramatic fluxes in carbon assimilation due to short growing seasons followed by long periods of winter dormancy. The subsequent fluctuations of carbon allocation from host to fungi may have cascading effects on soil carbon and nutrient cycling (Lloyd & Taylor 1994).

*Correspondence author. School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townshend Dr., Houghton, Michigan 49931, USA.
E-mail: shuppert@mtu.edu

© 2016 The Authors. Functional Ecology © 2016 British Ecological Society

Though ectomycorrhizal fungi depend on living hosts for carbon, their capacity for saprotrophism has recently been debated (Baldrian 2009; Cullings & Courty 2009; Bréda *et al*. 2013; Lindahl & Tunlid 2014). Ectomycorrhizal fungi (EMF) release a suite of extracellular enzymes to break down complex organic matter for nutrient acquisition in exchange for glucose derived from their plant hosts. Chitinases and phosphatases, for example, are released to degrade organic matter–protein complexes to acquire nitrogen and phosphorus, respectively (Pritsch & Garbaye 2011; Rineau *et al*. 2012). However, recent discoveries indicate that EMF also release enzymes which can decompose plant litter. Several findings have demonstrated that EMF secrete carbon-compound degrading enzymes to mobilize glucose (Courty, Bréda & Garbaye 2007; Cullings, Ishkhanova & Henson 2008; Courty, Franc & Garbaye 2010; Rineau *et al*. 2012), a trait historically only attributed to free-living saprotrophic fungi (Baldrian 2009). Moreover, laboratory studies have shown the potential of EMF to depolymerize carbon compounds for glucose acquisition, albeit at low rates compared to their saprotrophic counterparts (Burke, Smemo & Hewins 2014).

The purpose of direct decomposition of organic carbon compounds by EMF is debated, but two general models have been proposed. The first model, hereafter termed the 'nutrient acquisition model' (Talbot, Allison & Treseder 2008; Lindahl & Tunlid 2014; Moore *et al*. 2015), describes decomposition by EMF as a by-product of releasing nutrients locked in organic matter. Enzymes are secreted to break down carbon complexes in order to access nitrogen or phosphorus within the complexes, and carbon mobilization may simply be a secondary process, not the goal of decomposition. With this model, the level of enzyme secretion is not inversely dependent on carbon availability from the host tree, rather enzymes are secreted at a relatively consistent rate. The second model, hereafter termed the 'saprotrophy model' (Courty, Bréda & Garbaye 2007; Talbot, Allison & Treseder 2008; Courty, Franc & Garbaye 2010; Moore *et al*. 2015), proposes that the ability of EMF to decompose carbon compounds is inversely related to carbon allocation from the host tree. When allocation is high, enzymes for decomposition decrease and carbon mobilization from soil decreases. Likewise, when allocation is low, enzymes for decomposition increase, and carbon mobilization from soil increases. Due to the important role of EMF in soil carbon sequestration (Clemmensen *et al*. 2013; Averill, Turner & Finzi 2014), saprotrophism would represent a large carbon loss often misattributed in global carbon models (Moorhead & Sinsabaugh 2006; Allison 2012; Treseder *et al*. 2012).

Under the saprotrophy model, decomposition by EMF may be driven by the phenology of the host trees and the associated changes in carbon inputs to their symbionts. Deciduous trees in boreal forests spend most of the year dormant followed by a relatively short period for leaf flush, expansion and abscission – phenological stages which may directly influence the host–symbiont relationship as well as leaf litter input. Many tree species reserve sugar and starch for metabolism when photosynthetically derived carbon is limited. These sugar and starch reserves, collectively termed nonstructural carbohydrates (NSCs), fluctuate in response to seasonality and subsequently induce changes in source-sink dynamics in trees (Chapin, Schulze & Mooney 1990). For example, when Landhäusser & Lieffers (2003) monitored the fine root sugar and starch content of aspen (*Populus tremuloides* Michx.) from spring thaw to autumn frost, they found starch reserves initially low but increasing as the growing season progressed. Following leaf abscission however, starch levels dramatically declined towards the winter dormancy. Sugar concentrations were inversely related, with high levels observed during thaw and bud flush, decreasing as the growing season progressed, but increasing again during leaf abscission and ground frost. This pattern, observed in many tree species (Gruber, Pirkebner & Oberhuber 2013; Da Silva *et al*. 2014; Dang *et al*. 2014), may determine how much sugar is allocated to ectomycorrhizal fungi (Johansson 1993; Hoch, Richter & Körner 2003).

The mechanism of sugar transfer from host to EMF is poorly understood, but may be indirectly measured by monitoring invertase activity (Salzer & Hager 1993; Parrent *et al*. 2009). This plant-derived enzyme is secreted into the interfacial apoplast where it hydrolyses exuded sucrose into glucose and fructose, both of which can be absorbed by EMF; however, glucose is preferred (Smith & Read 2008). Most EMF lack genes encoding invertase and are unable to absorb sucrose; therefore, they rely on the host for invertase synthesis and sucrose hydrolysis (Salzer & Hager 1993; Schaeffer *et al*. 1995). Hosts may consequently control the amount of photosynthate allocated to associated symbionts by regulating invertase activity. As seasonality may cause fluctuations in fine root NSC reserves, invertase levels may also fluctuate, determining how much glucose EMF are receiving from their host.

When carbon is available, by processes represented by either the saprotrophy model or the nutrient acquisition model, EMF are able to forage for nutrients. Foraging strategy is often dependent on physical and functional characteristics of emanating hyphae, and emphasis has recently been placed on EMF exploration type, rather than lineage, to better indicate functional characteristics (Hobbie & Agerer 2010; Peay, Kennedy & Bruns 2011; Tedersoo *et al*. 2012; Fernandez & Kennedy 2015). Agerer (2001) was the first to classify EMF into exploration types based on the presence and length of emanating hyphae: contact types are characterized by a smooth mantle with few to no emanating hyphae, while medium- and long-distance types have increasing rhizomorph lengths. Exploration type may predict foraging patterns of EMF species in addition to extracellular enzyme secretions (Tedersoo *et al*. 2012; Lindahl & Tunlid 2014). For example, many contact types have been found to secrete greater levels of lignin-degrading enzymes while most long-distance

exploration types have been found to secrete greater levels of cellulose-degrading enzymes, which can be explained by their origin within saprotrophic groups of white rot fungi and brown rot fungi, respectively (Hibbett & Matheny 2009; Tedersoo *et al.* 2012; Burke, Smemo & Hewins 2014). The composition and seasonal turnover of EMF exploration types may therefore determine the set of functions performed by an EMF community (Courty *et al.* 2008; Rudawska, Leski & Stasińska 2011; Tedersoo *et al.* 2012; Clemmensen *et al.* 2015) and may have distinct responses to changes in host phenology.

The relationship among the phenology-dependent storage of NSCs, root invertase activity and EMF-secreted carbon-compound degrading enzymes is not well understood, but we anticipate these processes to be intimately linked due to the interdependence between trees and ectomycorrhizal fungi. The objective of this study was to quantify the potential carbon-compound degrading ability of *Populus tremuloides* ectomycorrhizas from host dormancy through leaf abscission. We tested whether phenological shifts in fine root carbon reserves of mature *P. tremuloides* affect the activity of EM-derived carbon-compound degrading enzymes, predicting that decomposing abilities of EMF would be dependent on host phenology and follow the saprotrophy model. Specifically, we hypothesized that root NSC reserves and invertase activity would be lowest during leaf flush and leaf abscission, when host photosynthesis is low but EMF are active. We predicted that in response, EMF-secreted carbon-compound degrading enzymes would be highest during these phenological stages and lowest during host dormancy and full leaf expansion.

Table 1. Total basal area index, *Populus tremuloides* basal area, and per cent *P. tremuloides* for eight mature *P. tremuloides* stands in northern Alberta, Canada, of approximately one hectare each

Site	Total basal area (m^{-2} ha^{-1})	*P. tremuloides* basal area (m^{-2} ha^{-1})	*P. tremuloides* (%)
1	216·81 (±2·33)	194·75 (±6·19)	89·82 (±3·82)
2	202·54 (±9·95)	202·54 (±9·95)	100
3	222·03 (±3·36)	220·52 (±3·89)	99·32 (±0·25)
4	168·96 (±10·42)	159·71 (±11·64)	94·53 (±1·08)
5	217·64 (±9·38)	212·96 (±11·57)	97·85 (±1·11)
6	200·73 (±6·45)	200·73 (±6·45)	100
7	192·49 (±5·43)	191·70 (±5·45)	99·59 (±0·02)
8	244·72 (±8·60)	244·68 (±8·61)	99·99 (±0·01)

Materials and methods

SITE DESCRIPTION

To test the relationship between host phenology and the activity of EMF-derived carbon-compound degrading enzymes, eight mature stands (~64 years old) of aspen (*Populus tremuloides*) were chosen near Conklin, north-eastern Alberta, Canada (55°38′N, 111°07′W) within the boreal mixedwood forest. Sites were chosen to be approximately 1 hectare in size and separated by at least 500 m, up to several kilometres. Aspen basal area averaged 98%, ranging from a minimum of 90% to 100% of total stand basal area (Table 1). Sites have a *Viburnum edule* (Michx.) Raf.-*Rosa acicularis* Lindl. understorey and Orthic Gray Luvisol soils. Mean precipitation for the area is 419 mm with a mean high air temperature of 16·8 °C in July and mean low temperature of −18·8 °C in January (1981–2010, Fig. 1). During each collection, roots were harvested for nonstructural carbohydrate (NSC) concentration, potential invertase activity and EMF-derived extracellular enzyme activity.

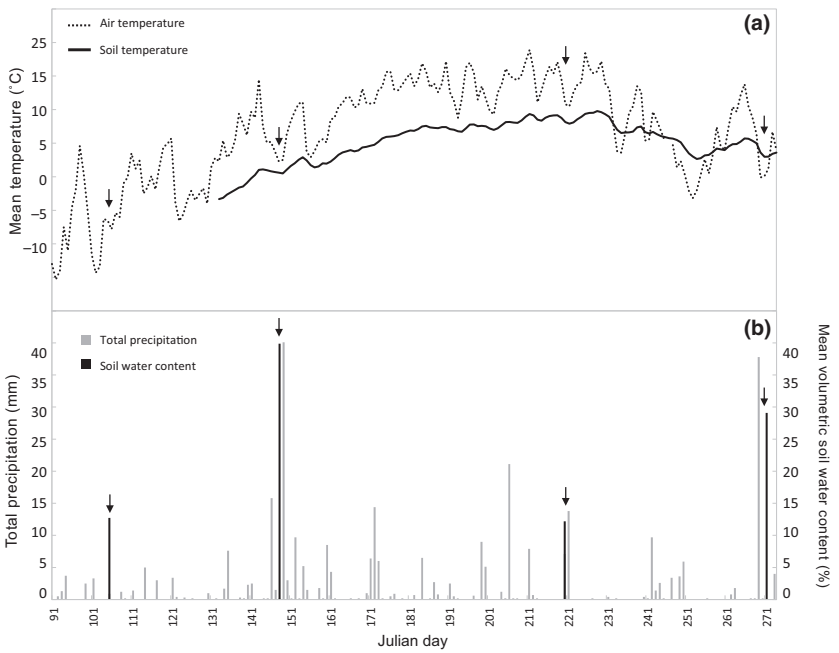

Fig. 1. (a) Mean daily air temperature (°C) for the Conklin, Alberta, Canada area and mean daily soil temperature (°C) for the eight mature *Populus tremuloides* stands. Air temperature was retrieved from the nearest weather station in Fort McMurray, Alberta, Canada. (b) Total precipitation (mm) for the Conklin, Alberta, Canada area and volumetric water content (%) for the eight mature *P. tremuloides* stands. Total precipitation was retrieved from the nearest weather station in Fort McMurray, Alberta, Canada. Arrows indicate sampling dates.

FIELD SAMPLING

Collection times of ectomycorrhizas and fine roots were determined by the phenological stage of the host tree. The four stages for collection were during: (i) tree dormancy and soil partially frozen (14/15 April 2014); (ii) leaf flush (27/28 May 2014); (iii) late growing season (7/8 August 2014); and (iv) leaf abscission (27/28 September 2014). Phenological stages were determined by visual observations.

To measure EMF-derived extracellular enzyme activity, fine roots were harvested from three mature trees by digging four holes in the four cardinal directions, approximately 15 cm deep and within 0·5 m of the host stem. For each of three trees, fine roots, traced to the host tree, were collected and pooled. Roots were stored with the surrounding soil in a plastic bag and placed on ice. To measure potential invertase activity, additional fine roots were collected from one mature tree also used for EMF-derived enzyme root collection, stored in separate plastic bags without surrounding soil and immediately placed on dry ice upon harvesting. For NSC analysis, fine root (≤2 mm diameter) samples were collected from the same mature aspen used for invertase activity for each site ($n = 8$). Roots of approximately 20 cm long were harvested within 0·5 m of the stem and immediately placed on ice. All samples were transported within 48 h to the University of Alberta. Roots for measuring EMF-derived extracellular enzyme activity were stored at 4–6 °C, while roots for invertase enzyme and NSC analyses were stored at −20 °C, and all roots collected for enzyme analyses processed within 28 days.

Soil temperature and volumetric soil water content were also recorded at each site. During the dormancy stage, soils were frozen and soil temperature was recorded using a UE DT130 digital thermometer with Omega Probe (Universal Enterprises, Inc., Beaverton, OR, USA) at a depth of 10 cm. After soil had thawed, three Hobo temperature pendants (Onset Computer Corporation, Bourne, MA, USA) were installed at each site at a depth of 10 cm, approximately 25 m apart recording temperature at 2-h intervals. Soil temperature ranged from a minimum of 0 °C during host dormancy to a maximum of 14·50 °C during full leaf expansion (Fig. 1).

Volumetric soil water content was measured around a target tree for each site, at a depth of 6 cm using a Theta Probe Soil Moisture Sensor (Delta-T Devices Ltd., Cambridge UK), at each cardinal direction approximately 0·5 m from the stem. Volumetric soil water content ranged from a minimum of 4·6% during full leaf expansion to a maximum of 53·4% during leaf flush. The mean soil water content was 12·7% (±1·10) during host dormancy, 39·9% (±2·25) during leaf flush, 12·2% (±1·24) during full leaf expansion and 29·1% (±0·82) during leaf abscission. Collections of roots during leaf flush and leaf abscission took place during periods of high precipitation in the area, which likely increased soil moisture (Fig. 1).

FINE ROOT NONSTRUCTURAL CARBOHYDRATES

Fine roots (≤2 mm) were thawed and gently washed over a 1·2-mm sieve to remove all soil and debris and subsequently oven-dried for 1 h at 75 °C followed by 60 °C for one week. After drying, roots were weighed to calculate mass, then ground through a 40-μm mesh screen in a Wiley mill. Starch and sugar concentrations were then measured following the protocol of Chow & Landhausser (2004). Briefly, sugars and starch were extracted with 80% hot ethanol. Sugar concentration was subsequently measured colorimetrically using phenol–sulphuric acid with a 2% phenol concentration. For starch concentrations, an enzyme digestion mixture of 1000 U α-amylase and 5 U amyloglucosidase was used on plant tissue samples. A peroxidase–glucose oxidase/*o*-dianisidine reagent was used to measure glucose hydrolysate obtained, followed by the addition of sulphuric acid and measured colorimetrically.

ROOT INVERTASE AND POTENTIAL ACTIVITY OF ECTOMYCORRHIZAL EXTRACELLULAR ENZYMES

Harvested roots were gently washed with tap water over a 1·2-mm sieve to remove soil and debris, and cut into 1- to 2-cm fragments. Root fragments were mixed thoroughly in a container filled with deionized water using forceps. Root tips were placed in a petri dish with deionized water and examined under a dissecting microscope at 100× magnification. Ectomycorrhizal root tips were morphotyped by appearance of hyphae, mantle structure, colour and texture (Goodman *et al*. 1996).

Assays of root invertase activity were performed on up to 75 mycorrhizal root tips per site using materials and methods provided by Sigma-Aldrich (Invertase Assay Kit, Catalog Number MAK118, Sigma-Aldrich, St. Louis, MO, USA). Briefly, root tips were placed in separate wells of a 96-well plate. Each well received 5 μL of a 1× sucrose solution and dark incubated at 30 °C for 20 min. Following incubation, a solution of buffer, enzyme mix and dye reagent, which fluoresces in reaction with glucose, was added to each well and dark incubated again for 20 min at room temperature. Additional details are provided in Appendix S1 of Supporting Information.

For ectomycorrhizal enzyme assays, a maximum of seven roots tips were collected for up to four EMF morphotypes per tree, for three trees per site, corresponding to a maximum of 84 root tips assayed per site. Activity of four enzymes was measured: β-glucuronidase, β-glucosidase, *N*-acetylglucosaminidase and laccase. β-glucuronidase, a hemicellulase, hydrolyses the bond between glucuronic acid and an organic complex, releasing glucuronic acid for further degradation to glucose. β-glucosidase is a cellulase which degrades plant cell wall material by hydrolysing the bond between two glucose molecules. A chitinase, *N*-acetylglucosaminidase releases nitrogen by hydrolysing the glycosidic bonds in chitin. Laccase contributes to plant cell wall decomposition by oxidizing the linked phenols in lignin.

Ectomycorrhizal enzyme assays were conducted using the procedures described by Pritsch *et al*. (2011). Briefly, solutions of 4-methylumbelliferone (MUB) and diammonium 2,2′-azinobis-3-ethylbenzothiazoline (ABTS) were made for the assay substrates, corresponding to the fluorometric assay used for β-glucuronidase, β-glucosidase and *N*-acetylglucosaminidase, and colorimetric assay for laccase, respectively. Root tips were incubated at room temperature for 15 min (β-glucosidase and *N*-acetylglucosaminidase), 30 min (β-glucuronidase) or 60 min (laccase). Following root invertase and ectomycorrhizal enzyme assays, root tips were scanned and projected area was measured with WINRHIZO PRO 2009b software (Regent Instruments Inc., Québec, Canada). Further details are provided in Appendix S1.

IDENTIFICATION OF ECTOMYCORRHIZAL FUNGI

Ectomycorrhizal morphotypes were classified as either 'contact' or 'distance' exploration types based on the presence and length of emanating hyphae (Table S1). These two broad categories were chosen to eliminate inconsistencies in mycelial length observations. Once assays were completed, two specimens of each morphotype per tree were collected for DNA extraction and identification, for a total of up to twenty-four root tips per site. Fungal DNA of colonized root tips selected for molecular analysis was extracted, and the ITS region was amplified using the fungal-specific ITS1-F and ITS4 primer pair (Innis *et al*. 1990; Gardes & Bruns 1993). Amplification was confirmed by gel electrophoresis, and then, amplified product was purified and a bidirectional sequencing reaction was

performed and subsequently read by an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Raw sequences were edited with GENEIOUS software (BioMatters, Auckland, New Zealand), clustered into operational taxonomic units and run through the GenBank data base (National Center for Biotechnology Information, Bethesda, MD, USA) using BLASTN to identify the best match. Identity was assigned to an OTU if per cent identity was ≥97 and query coverage was ≥80%. Additional details of extraction, amplification, sequencing and editing are available in Appendix S1.

STATISTICAL ANALYSES

Nonstructural carbohydrate (NSC) concentration, root invertase activity and potential enzyme activity were averaged for each site ($n = 8$), and the effect of phenological stage was tested with one-way ANOVAs. Values were transformed as necessary to meet assumptions of normality prior to analysis. Significant effects ($P < 0{\cdot}05$) were followed by Tukey tests to examine differences among phenological stages ($\alpha = 0{\cdot}05$). Pearson correlation coefficients were calculated between potential enzyme activities, NSC concentrations, soil temperature and volumetric soil water content. The effects of ectomycorrhizal exploration type, host phenology and their interaction on potential enzyme activity were tested using a two-way ANOVA when assumptions were met, and a permutational ANOVA when assumptions were not met. The effect of phenological stage on the ratio of contact to distance exploration types, and on the relative abundance of contact and distance exploration types, was tested with a one-way ANOVA. Significant effects were followed by Tukey tests to examine differences among phenological stages ($\alpha = 0{\cdot}05$). All statistical tests were performed with R software (R Development Core Team 2011).

Results

HOST PHENOLOGY AND ROOT NONSTRUCTURAL CARBOHYDRATE STATUS

Phenology affected the total nonstructural carbohydrate (NSC; soluble sugars and starch) concentration of fine roots (Table 2, Fig. 2). Specifically, mean total NSC concentration (±1 standard error) of fine roots was 6·0% (±0·61) during host dormancy, increased to 10·1% (±0·39) during leaf flush, further increasing to 13·9% (±0·54) during full leaf expansion, which was maintained during leaf abscission (14·1 ± 0·59). Each of the components of NSCs followed a similar trend. Fine root sugar concentrations were affected by phenology ($P < 0{\cdot}001$, Table 2, Fig. 2), with sugar concentration increasing from 5·7% (±0·56) during host dormancy to 9·0% (±0·34) during leaf flush and remaining constant across the remaining phenological stages (8·7% ± 0·44 and 9·3% ± 0·34, respectively). Over the same phenological stages fine root starch concentrations increased from 0·3% (±0·07) during host dormancy to 1·2% (±0·09) during leaf flush, to approximately 5·0% (±0·53) during full leaf expansion and 4·8% (±0·37) during leaf abscission stages ($P < 0{\cdot}001$, Table 2, Fig. 2). Sugar concentration of fine roots was positively correlated with soil temperature ($r^2_{(8)} = 0{\cdot}55$, $P < 0{\cdot}001$, Table 3) and soil water content ($r^2_{(8)} = 0{\cdot}44$, $P < 0{\cdot}05$, Table 3). Starch was positively correlated with soil temperature ($r^2_{(8)} = 0{\cdot}82$, $P < 0{\cdot}001$, Table 3).

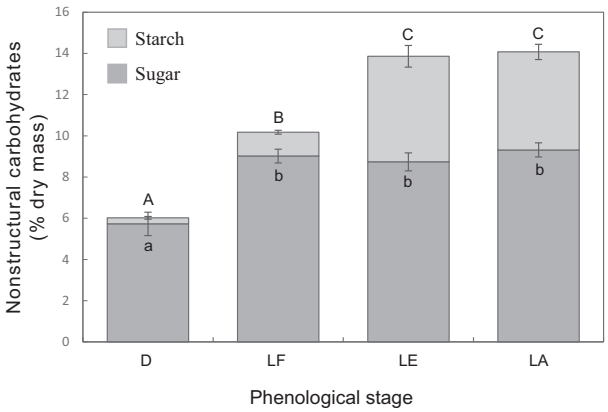

Fig. 2. Mean nonstructural carbohydrate concentration of fine roots collected from mature *Populus tremuloides* stands ($n = 8$) during four phenological stages (D, dormancy; LF, leaf flush; LE, full leaf expansion; and LA, leaf abscission). Across phenological stages, upper- and lower-case letters denote significant differences among means (±1 standard error) of starch and sugar concentrations, respectively ($\alpha = 0{\cdot}05$).

Table 2. Analysis of variance for the effect of host phenological stage on total nonstructural carbohydrate concentration, sugar concentration and starch concentration. Fine roots were collected from mature *Populus tremuloides* stands ($n = 8$) in northern Alberta, Canada. Host phenological stage includes dormancy, leaf flush, full leaf expansion and leaf abscission

Source	d.f.	Sum. Sq	*F*-value	*P*-value
Total NSC*	3	344·800	47·240	<0·0001
Residuals	21	51·100	2·430	
Sugar†	3	66·520	13·91	<0·0001
Residuals	21	33·480	1·594	
Starch‡	3	13·630	4·543	<0·0001
Residuals	21	0·771	0·037	

d.f., degrees of freedom; Sum Sq, sum of squares.
*Nonstructural carbohydrate concentration (percent dry mass).
†Sugar concentration (percent dry weight).
‡Starch concentration (percent dry weight).

ROOT INVERTASE ACTIVITY

Host phenology affected root invertase activity (Table 4, Fig. 3). Specifically, the mean (±1 standard error) potential root invertase activity was 125·1 pmol mm^{-2} min^{-1} (±28·6) during host dormancy, which increased to 190·0 pmol mm^{-2} min^{-1} (±46·7) during leaf flush, and after that did not change significantly during stages of full leaf expansion and leaf abscission with 151·3 pmol mm^{-2} min^{-1} (±23·3) and 210·5 pmol mm^{-2} min^{-1} (±21·8), respectively. Root invertase activity was positively correlated with fine root sugar concentrations ($r^2_{(8)} = 0{\cdot}49$, $P < 0{\cdot}01$, Table 3), soil temperature ($r^2_{(8)} = 0{\cdot}38$, $P < 0{\cdot}05$, Table 3) and soil water content ($r^2_{(8)} = 0{\cdot}42$, $P < 0{\cdot}01$, Table 3).

Table 3. Pearson correlation coefficients among potential enzyme activities of ectomycorrhizal root tips, fine root sugar concentrations and soil conditions across four phenological stages (dormancy, leaf flush, full leaf expansion and leaf abscission) of *Populus tremuloides*. Roots were collected from mature *P. tremuloides* stands in northern Alberta, Canada. Bold values denote a significant correlation ($P < 0{\cdot}05$, $n = 8$ sites). Bold, italic values denote a marginally significant correlation ($P < 0{\cdot}10$)

	Gls	Glr	Nag	Lac	Inv	Sugar	Starch	Temp
Gls								
Glr	***0·34***							
Nag	**0·68**	0·19						
Lac	**0·54**	0·17	**0·45**					
Inv	−0·14	−0·21	−0·08	0·11				
Sugar	**−0·47**	−0·18	−0·22	−0·10	**0·49**			
Starch	−0·17	−0·10	0·16	0·18	0·27	**0·47**		
Temp	−0·19	−0·20	0·17	0·00	**0·38**	**0·55**	**0·82**	
SWC	**−0·31**	**−0·42**	−0·29	−0·22	**0·42**	**0·44**	−0·15	−0·08

Gls, β-glucosidase; Glr, β-glucuronidase; Nag, *N*-acetylglucosaminidase; Lac, laccase; Inv, root invertase; Temp, soil temperature; SWC, soil water content.

Table 4. Analysis of variance for the effect of host phenological stage on potential enzyme activity of ectomycorrhizal root tips collected from mature *Populus tremuloides* stands ($n = 8$) in northern Alberta, Canada. Host phenological stage includes dormancy, leaf flush, full leaf expansion and leaf abscission

Source	d.f.	Sum. Sq	Mean Sq.	*F*-value	*P*-value
β-glucuronidase	3	1·199	0·400	3·269	0·042
Residuals	21	2·566	0·122		
β-glucosidase	3	23·56	7·852	1·308	0·298
Residuals	21	126·04	6·002		
N-acetylglucosaminidase	3	96·0	31·990	1·298	0·301
Residuals	21	517·6	24·650		
Laccase	3	718766	260589	2·586	0·080
Residuals	21	2115805	100753		
Invertase	3	17·538	5·846	12·82	<0·0001
Residuals	21	9·573	0·456		

d.f., degrees of freedom; Sum Sq, sum of squares; Mean Sq, mean square.

POTENTIAL ECTOMYCORRHIZAL ENZYME ACTIVITIES

Of the measured enzymes secreted by ectomycorrhizas, host phenology had the largest effect on the activity of β-glucuronidase (Table 4). The most pronounced difference in the activity of β-glucuronidase was between dormancy and leaf flush (Fig. 3). Potential activities of β-glucosidase, *N*-acetylglucosaminidase and laccase were not significantly affected by host phenology; however, the effect size is uncertain due to large variation. (Table 4, Fig. 3). Potential activities of β-glucosidase, *N*-acetylglucosaminidase and laccase were positively correlated (Table 3), while fine root sugar concentration and activities of β-glucosidase were negatively correlated (Table 3). Despite a trend showing an inverse relationship between β-glucuronidase and invertase (Fig. 3), there was no significant correlation (Table 3).

TAXA AND EXPLORATION TYPES OF ECTOMYCORRHIZAL FUNGI

Across the four phenological stages, DNA of 62 enzyme-assayed root tips was amplified with a success rate of 60%. The DNA of the resulting 37 root tips was sequenced and quality filtering yielded 26 sequences clustered into four operational taxonomic units (OTUs) and 14 remaining singletons (Table S2). In total, 18 ectomycorrhizal fungal taxa were recorded; the most common taxa were *Russula* occurring at all phenological stages, and *Cenococcum geophilum* which was observed during three of the four phenological stages (Fig. 4). When ectomycorrhizal root tips were categorized by exploration type, we found that exploration type affected potential activity of all enzymes but β-glucosidase (Table 5, Fig. S1). Overall, distance exploration types had higher potential activity of β-glucuronidase, particularly during the phenological stages other than leaf flush (Table 5, Fig. S1). Potential *N*-acetylglucosaminidase activity was also higher in distance exploration types (Table 5, Fig. S1). Activities of laccase were marginally higher in contact exploration types (Table 5, Fig. S1), but the high variation of β-glucosidase, *N*-acetylglucosaminidase and laccase activities from both exploration types masked any potential effect of phenological stage. Contact exploration types included OTUs belonging to the *Russula* genus (Morphotypes E4, E12), an uncultured *Thelephora* clone (Morphotype E10) and a *Phialocephala fortinii* strain

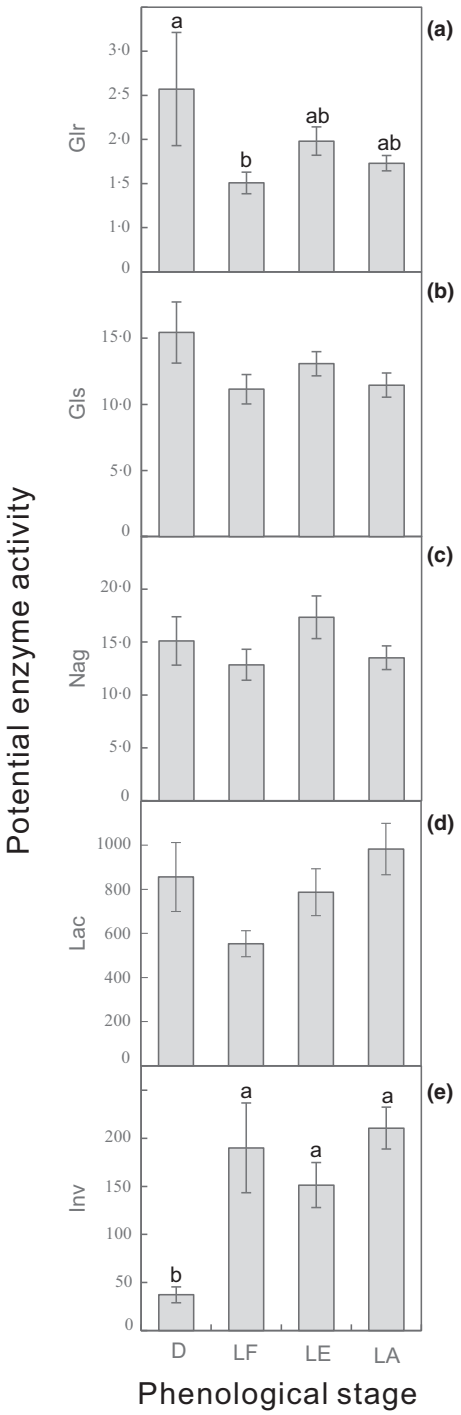

Fig. 3. Mean (±1 standard error) potential activity of (a) β-glucuronidase (Glr), (b) β-glucosidase (Gls), (c) *N*-acetylglucosaminidase (Nag), (d) laccase (Lac) and (e) root invertase (Inv) of ectomycorrhizal root tips across four phenological stages of mature *Populus tremuloides* (D, dormancy; LF, leaf flush; LE, full leaf expansion; and LA, leaf abscission). Ectomycorrhizal roots were collected from *P. tremuloides* stands ($n = 8$) in northern Alberta, Canada. Letters denote significant differences in activity among phenological stages ($\alpha = 0{\cdot}05$). All enzyme values are reported in (pmol mm^{-2} min^{-1}).

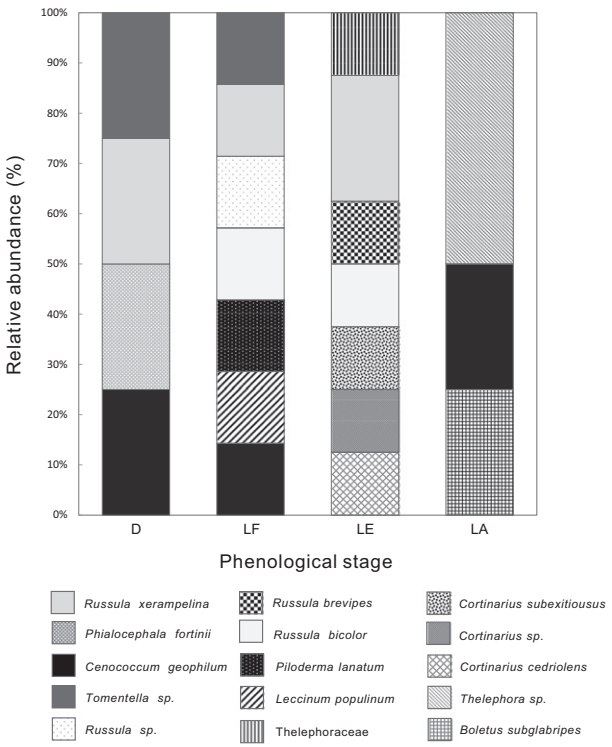

Fig. 4. Relative abundance of ectomycorrhizal fungal taxa. Roots were collected from mature *Populus tremuloides* stands ($n = 8$) in northern Alberta, Canada, during host dormancy (D), leaf flush (LF), full leaf expansion (LE) and leaf abscission (LA). Owing to differences in sequencing success, fungi forming ectomycorrhizas were not able to be identified at four and two stands during host dormancy and leaf abscission, respectively.

(E12, Table S1). Distance exploration types included OTUs matching sequences of a *Cenococcum geophilum* isolate (Morphotypes E1, E2), a *Russula* sp. isolate (Morphotype E2), a *Boletus subglabripes* isolate (Morphotype E2), a *Cortinarius cedriolens* isolate (Morphotype E2), an uncultured *Cortinarius* clone (Morphotype E2), a *Piloderma lanatum* clone (Morphotype E2), a *Leccinum populinum* isolate (E11) and a *Cortinarius subexitiosus* clone (Morphotype E11, Table S1).

The ratio of contact to distance exploration types did not change across phenological stages ($F_{(3,28)} = 0{\cdot}94$, $P = 0{\cdot}44$); however, phenological stage affected the relative abundance of distance exploration type ectomycorrhizal fungi ($P < 0{\cdot}01$, Table 6, Fig. 5). Leaf flush coincided with a lowered relative abundance of distance exploration types, but was unchanged across the other phenological stages. The relative abundance of contact exploration types remained similar across each phenological stage.

Discussion

We predicted host phenology to affect the activity of carbon-compound degrading enzymes by EMF due to changes in root nonstructural carbohydrates and correlated fluctuations in root invertase activity. Though root nonstructural carbohydrates and invertase activity changed seasonally, we found that aside from β-glucuronidase, the other carbon-compound degrading enzymes

Table 5. Effects of ectomycorrhizal exploration type, host phenology and their interaction on potential enzyme activity of ectomycorrhizal root tips collected from mature *Populus tremuloides* stands in northern Alberta, Canada ($n = 8$). Host phenological stages include dormancy, leaf abscission, full leaf expansion and leaf abscission. Exploration type includes contact and distance ectomycorrhizal fungi

Source	d.f.	Sum Sq	*F*-value	*P*-value
β-glucuronidase*				
Exploration type	1	0·679	5·084	0·028
Host phenology	3	2·256	5·633	0·002
Exploration type × host phenology	3	0·078	0·196	0·899
Residuals	56	7·476		
β-glucosidase*				
Exploration type	1	0·200	0·009	0·926
Host phenology	3	85·000	1·468	0·233
Exploration type × host phenology	3	8·500	0·147	0·931
Residuals	56	1080·500		
N-acetylglucosaminidase*				
Exploration type	1	1·636	5·399	0·024
Host phenology	3	0·923	1·015	0·393
Exploration type × host phenology	3	0·253	0·278	0·841
Residuals	56	16·973		
	d.f.	Chi-square		*P*-value
Laccase†				
Exploration type	1	3·739		0·053
Host phenology	3	3·034		0·387

d.f., degrees of freedom; Sum Sq, sum of squares.
*Calculated with two-way analysis of variance.
†Calculated with Kruskal–Wallis test.

Table 6. Effects of host phenological stage on the relative abundance of contact and distance ectomycorrhizal exploration types. Ectomycorrhizal root tips were collected from mature *Populus tremuloides* stands in northern Alberta, Canada ($n = 8$). Host phenological stages include dormancy, leaf abscission, full leaf expansion and leaf abscission

	d.f.	Sum. Sq	Mean Sq	*F*-value	*P*-value
Contact	3	0·014	0·005	0·269	0·847
Residuals	28	0·483	0·017		
Distance	3	0·272	0·091	6·011	0·003
Residuals	28	0·422	0·015		

d.f., degrees of freedom; Sum Sq, sum of squares; Mean Sq, mean square.

were unaffected; however, the high variability might have masked any potential effects of host phenological stage. Ectomycorrhizal exploration type was a stronger predictor of potential extracellular enzyme activity than phenology with distance exploration types having higher activity of two of the four enzymes. Moreover, the relative abundance of distance exploration types declined during leaf flush.

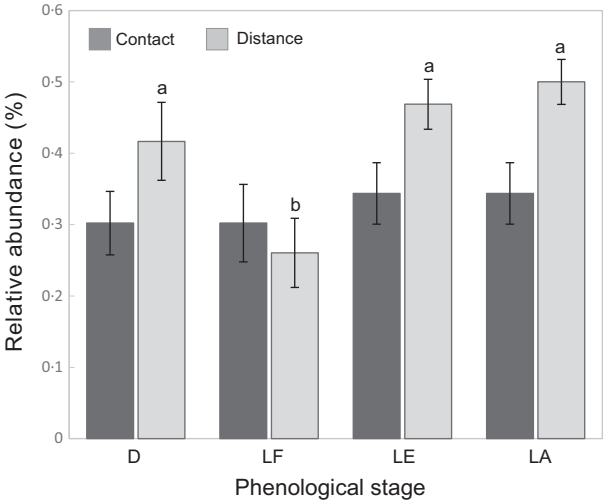

Fig. 5. Relative abundance of contact and distance ectomycorrhizal fungal exploration types across four phenological stages of mature *Populus tremuloides*. Phenological stages include dormancy (D), leaf flush (LF), full leaf expansion (LE) and leaf abscission (LA). Ectomycorrhizal roots were collected from mature *P. tremuloides* stands ($n = 8$) in northern Alberta, Canada. Letters denote significant ($\alpha = 0{\cdot}05$) differences in relative abundance among phenological stages.

POTENTIAL ECTOMYCORRHIZAL EXTRACELLULAR ENZYME ACTIVITY

Taken together, our results on potential EMF enzyme activity support the nutrient acquisition model, but are not unequivocal. Contrary to our prediction, we found only weak relationships between host phenology and overall activity of β-glucosidase, *N*-acetylglucosaminidase and laccase. Phenology affected activity of β-glucuronidase, which was highest during host dormancy and lowest during leaf flush. The high activity during dormancy may indicate a greater need by ectomycorrhizal fungi to mobilize glucose, as predicted by the saprotrophy model, or access nutrients locked within the organic compounds linked to β-glucuronidase, as predicted by the nutrient acquisition model. Courty, Bréda & Garbaye (2007) recorded a spike in potential β-glucuronidase activity of ectomycorrhizas shortly after leaf flush in a temperate oak forest and suggested its role in leaf development, a finding that could support the nutrient acquisition model. We found activity of β-glucuronidase to be marginally higher during full leaf expansion than leaf flush, perhaps a reflection of its role in nutrient acquisition during leaf maintenance throughout the growing season and supporting the nutrient acquisition model. On the other hand, defoliation has been found to induce elevated activity of β-glucosidase and laccase (Cullings, Ishkhanova & Henson 2008), suggesting that when the supply of photosynthate decreases, EMF are able to increase mobilization of soil carbon. We anticipated a similar outcome in response to leaf abscission, but found no strong effect.

Root invertase activity increased beyond host dormancy but was not significantly correlated with the

activity of any EMF-derived extracellular enzymes, a finding that supports the nutrient acquisition model but is not unequivocal. Leaf litter input during leaf abscission provides a rich supply of soluble sugars and organic matter for decomposition (Landhäusser & Lieffers 2003). Our initial hypothesis, based on the saprotrophy model, was that EMF-derived enzyme activity would be significantly higher during this period due to loss of photosynthetic leaf area, and subsequent reduced carbon allocation to root symbionts. However, we found that activity of most of the EMF-derived enzymes was relatively constant during the season, instead supporting the nutrient acquisition model.

The potential activities of β-glucuronidase and β-glucosidase were negatively correlated with volumetric soil water content, a finding that differs from previous work that found no correlation (Courty, Bréda & Garbaye 2007; Herzog *et al*. 2013). Our findings may indicate that as soil dries, the rates of enzyme diffusion in the soil decrease, which may require higher rates of enzyme secretion. On the other hand, lower soil temperatures in the spring and fall may inhibit proteolytic activity on enzymes, resulting in an accumulation of enzymes (Lee *et al*. 2007; Wallenstein, Mcmahon & Schimel 2009).

HOST PHENOLOGY AND NONSTRUCTURAL CARBOHYDRATES

We predicted enzyme activity of EMF to be explained by the seasonal changes in root NSC concentrations during different phenological stages of the host. Host fine root NSC concentrations were lowest during dormancy and increased during leaf flush and full leaf expansion while remaining high during leaf abscission. Although leaf abscission dramatically decreases photosynthesis, most NSC accumulation occurred earlier in the growing season, allowing trees to prepare for dormancy (Chapin, Schulze & Mooney 1990; Landhäusser & Lieffers 2003). Though fine root NSCs were not correlated to activity of most of the carbon-compound degrading enzymes, changes in root growth and its effects on NSC storage and demand may have influenced enzyme activity of EMF. In general, root growth and root NSC storage are lower in the spring than later in the season (Gruber, Pirkebner & Oberhuber 2013; Dang *et al*. 2014). During dormancy, root NSC storage is low and frozen soil likely results in low carbon export to EMF and subsequently higher activity of β-glucuronidase. During leaf flush, root sugar concentration is higher, but limited root growth may result in more carbon allocation to EMF and subsequently lower activity of β-glucuronidase. Higher root growth typically occurs after shoot expansion and until leaf abscission, creating a higher demand for NSCs, and subsequently lower carbon allocation to EMF. This may explain why activity of β-glucuronidase was slightly higher during these two stages when compared to leaf flush. On the other hand, we pooled enzyme activity from the roots of three trees per stand, while NSCs were only measure on one tree per stand, a procedure which may have affected our results.

ROOT INVERTASE ACTIVITY

We predicted root invertase activity to be positively correlated with fine root sugar concentrations. Our results support this hypothesis and suggest that higher fine root sugar concentration may translate into higher glucose levels in the plant–fungus interface. We also predicted host phenology to affect root invertase activity and found this to be true. Although we predicted invertase activity to change over time, we anticipated levels to be lowest during leaf abscission and highest during full leaf expansion. Our results may reflect the host's investment in EMF even during carbon-expensive periods such as leaf flush. This could benefit the host if EMF nutrient foraging remains high during this phenological stage (Smith & Read 2008). On the other hand, bacteria can secrete extracellular invertase (Parrent *et al*. 2009) and may have contributed to the values we observed if they were not effectively removed during sample preparation. Additionally, our method of measuring invertase activity from severed fine roots may have captured a large portion of intracellular invertases, which are important for plant growth and development (Egger & Hampp 1993). If that is the case, high invertase levels during leaf flush, full leaf expansion and leaf abscission may not necessarily reflect higher carbon delivery to fungi. However, if carbon export to EMF is diffusive, our method would be an accurate proxy for detecting the level of glucose becoming available in the interfacial apoplast for EMF uptake.

ECTOMYCORRHIZAL EXPLORATION TYPES

The relative abundance of ectomycorrhizal exploration types varied during the growing season, perhaps as a direct result of changes in host carbon inputs. The abundance of distance exploration types was significantly lower during leaf flush, while there was no change in the abundance of contact exploration types. Rhizomorphs and emanating mycelium require a greater carbon investment by the host than contact exploration types (Agerer 2001). Consequently, during carbon-expensive periods such as leaf flush, the host may conserve NSCs and in turn allocate less carbon to distance exploration types, a finding consistent with defoliation studies (Saikkonen *et al*. 1999; Saravesi *et al*. 2008).

We found exploration type to be a better predictor of EMF-derived extracellular enzyme activity than host phenological stage. Potential activity of β-glucuronidase was higher in distance types, a finding consistent with previous work (Tedersoo *et al*. 2012). We also found potential activity of *N*-acetylglucosaminidase higher in distance exploration types, suggesting greater chitin-degrading abilities than in contact exploration types. Similarly, Hobbie & Agerer (2010) found that high-biomass mycorrhizas, that

is long-distance exploration types, had greater sporocarp ^{15}N enrichment than low-biomass mycorrhizas, perhaps further demonstrating enhanced nitrogen foraging characteristics of distance exploration types. We found contact exploration types to have marginally higher potential laccase activity than distance exploration types, a finding consistent with previous work (Tedersoo *et al.* 2012). For example several *Russula* species, a contact exploration type present on our roots, have recorded greater phenol oxidase (laccase) secretions in similar studies (Luis *et al.* 2005; Courty, Franc & Garbaye 2010; Burke, Smemo & Hewins 2014). The higher levels of potential laccase activity in Russulaceae belonging to contact exploration types gives them an important role in decomposing phenol–protein complexes, while the great taxonomic diversity within distance exploration types may have an important role in decomposing hemicelluloses and chitin. A diverse set of exploration types could therefore supply the host with various specific benefits in nutrient acquisition.

In conclusion, only the potential activity of β-glucuronidase coincided with host phenology, but not in the pattern we predicted based on the saprotrophy model. Observed trends warrant further investigation into the biotrophy–saprotrophy continuum; however, our evidence suggests that the secretion of carbon-compound degrading enzymes by EMF is likely driven by nutrient acquisition. The enzymes we measured may be utilized in tandem by EMF to break apart organic material in order to acquire nitrogen or phosphorus locked within carbon compounds. In addition, exploration type of EMF determined the potential activity of β-glucuronidase, *N*-acetylglucosaminidase and, to a smaller extent, laccase. Distance exploration types had higher potential activity of β-glucuronidase and *N*-acetylglucosaminidase, while contact exploration types had marginally higher potential laccase activity. Differences in potential enzyme activity among contact and distance exploration types throughout phenological stages point to unique functional roles which may change seasonally. These functional roles necessitate further investigation in order to better predict temporal patterns of carbon and nutrient cycling in boreal forests.

Acknowledgements

We thank Pak Chow, Fran Leishman, Cheryl Nargang and many members of the Landhäusser research group for technical support. We would also like to thank Erin Wiley for providing helpful comments. S.F.H. was supported by grants from National Science and Engineering Research Council of Canada (NSERC) and the Alberta Conservation Association.

Conflict of interest

None of the authors have a conflict of interest to declare.

Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.ht4t3 (Hupperts *et al.* 2016).

Fungal DNA sequences have been deposited in GenBank.

References

Agerer, R. (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza*, **11**, 107–114.

Allison, S.D. (2012) A trait-based approach for modelling microbial litter decomposition. *Ecology Letters*, **15**, 1058–1070.

Averill, C., Turner, B.L. & Finzi, A.C. (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, **505**, 543–546.

Baldrian, P. (2009) Ectomycorrhizal fungi and their enzymes in soils: is there enough evidence for their role as facultative soil saprotrophs? *Oecologia*, **161**, 657–660.

Bréda, N., Maillard, P., Montpied, P., Bréchet, C., Garbaye, J. & Courty, P.-E. (2013) Isotopic evidence in adult oak trees of a mixotrophic lifestyle during spring reactivation. *Soil Biology and Biochemistry*, **58**, 136–139.

Burke, D.J., Smemo, K.A. & Hewins, C.R. (2014) Ectomycorrhizal fungi isolated from old-growth northern hardwood forest display variability in extracellular enzyme activity in the presence of plant litter. *Soil Biology and Biochemistry*, **68**, 219–222.

Chapin, F.S., Schulze, E.-D. & Mooney, H.A. (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics*, **21**, 423–447.

Chow, P.S. & Landhausser, S.M. (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology*, **24**, 1129–1136.

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H. *et al.* (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, **339**, 1615–16188.

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J. & Wardle, D.A. (2015) Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist*, **205**, 1525–1536.

Courty, P.-E., Bréda, N. & Garbaye, J. (2007) Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. *Soil Biology and Biochemistry*, **39**, 1655–1663.

Courty, P.-E., Franc, A. & Garbaye, J. (2010) Temporal and functional pattern of secreted enzyme activities in an ectomycorrhizal community. *Soil Biology and Biochemistry*, **42**, 2022–2025.

Courty, P.-E., Franc, A., Pierrat, J.-C. & Garbaye, J. (2008) Temporal changes in the ectomycorrhizal community in two horizons of a temperate oak forest. *Applied and Environmental Microbiology*, **74**, 5792–5801.

Cullings, K. & Courty, P.-E. (2009) Saprotrophic capabilities as functional traits to study functional diversity and resilience of ectomycorrhizal community. *Oecologia*, **161**, 661–664.

Cullings, K., Ishkhanova, G. & Henson, J. (2008) Defoliation effects on enzyme activities of the ectomycorrhizal fungus *Suillus granulatus* in a *Pinus contorta* (lodgepole pine) stand in Yellowstone National Park. *Oecologia*, **158**, 77–83.

Da Silva, D., Qin, L., Debuse, C. & Dejong, T.M. (2014) Measuring and modelling seasonal patterns of carbohydrate storage and mobilization in the trunks and root crowns of peach trees. *Annals of Botany*, **114**, 643–652.

Dang, H.S., Zhang, K.R., Zhang, Q.F. & Xu, Y.M. (2014) Temporal variations of mobile carbohydrates in *Abies fargesii* at the upper tree limits. *Plant Biology*, **17**, 106–113.

Egger, B. & Hampp, R. (1993) Invertase, sucrose synthase and sucrose phosphate synthase in lyophilized spruce needles; microplate reader assays. *Trees*, **7**, 98–103.

Fernandez, C.W. & Kennedy, P.G. (2015) Moving beyond the black-box: fungal traits, community structure, and carbon sequestration in forest soils. *New Phytologist*, **205**, 1378–1380.

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology*, **2**, 113–118.

Goodman, D.M., Durall, D.M., Trofymow, J.A. & Berch, S.M. (1996) *A Manual of Concise Descriptions of North American Ectomycorrhizae*. Mycologue Publications, Victoria, BC, Canada.

Gruber, A., Pirkebner, D. & Oberhuber, W. (2013) Seasonal dynamics of mobile carbohydrate pools in phloem and xylem of two alpine timberline conifers. *Tree Physiology*, **33**, 1076–1083.

Herzog, C., Peter, M., Pritsch, K., Günthardt-Goerg, M.S. & Egli, S. (2013) Drought and air warming affects abundance and exoenzyme profiles of *Cenococcum geophilum* associated with *Quercus robur*, *Q. petraea* and *Q. pubescens*. *Plant Biology*, **15**, 230–237.

Hibbett, D.S. & Matheny, P.B. (2009) The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. *BMC Biology*, **7**, 1–13.

Hobbie, E.A. & Agerer, R. (2010) Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. *Plant and Soil*, **327**, 71–83.

Hoch, G., Richter, A. & Körner, C. (2003) Non-structural carbon compounds in temperate forest trees. *Plant Cell and Environment*, **26**, 1067–1081.

Hupperts, S.F., Karst, J., Pritsch, K. & Landhäusser, S.M. (2016) Data from: Host phenology and potential saprotrophism of ectomycorrhizal fungi in the boreal forest. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.ht4t3

Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (1990) *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, CA.

Johansson, T. (1993) Seasonal changes in contents of root starch and soluble carbohydrates in 4–6-year old *Betula pubescens* and *Populus tremula*. *Scandinavian Journal of Forest Research*, **8**, 94–106.

Landhäusser, S.M. & Lieffers, V.J. (2003) Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. *Trees*, **17**, 471–476.

Lee, Y.B., Lorenz, N., Dick, L.K. & Dick, R.P. (2007) Cold storage and pretreatment incubation effects on soil microbial properties. *Soil Biology and Biochemistry*, **71**, 1299–1305.

Lindahl, B.D. & Tunlid, A. (2014) Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytologist*, **205**, 1443–1447.

Lloyd, J. & Taylor, J.A. (1994) On the temperature dependence of soil respiration. *Functional Ecology*, **8**, 315–323.

Luis, P., Kellner, H., Zimdars, B., Langer, U., Martin, F. & Buscot, F. (2005) Patchiness and spatial distribution of laccase genes of ectomycorrhizal, saprotrophic, and unknown basidiomycetes in the upper horizons of a mixed forest cambisol. *Microbial Ecology*, **50**, 570–579.

Moore, J.A.M., Jiang, J., Post, W.M. & Classen, A.T. (2015) Decomposition by ectomycorrhizal fungi alters soil carbon storage in a simulation model. *Ecosphere*, **6**, 1–16.

Moorhead, D.L. & Sinsabaugh, R.L. (2006) A theoretical model of litter decay and microbial interaction. *Ecological Monographs*, **76**, 151–174.

Parrent, J.L., James, T.Y., Vasaitis, R. & Taylor, A.F.S. (2009) Friend or foe? Evolutionary history of glycoside hydrolase family 32 genes encoding for sucrolytic activity in fungi and its implications for plant-fungal symbioses. *BMC Evolutionary Biology*, **9**, 1–16.

Peay, K.G., Kennedy, P.G. & Bruns, T.D. (2011) Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? *Fungal Ecology*, **4**, 233–240.

Pritsch, K. & Garbaye, J. (2011) Enzyme secretion by ECM fungi and exploitation of mineral nutrients from soil organic matter. *Annals of Forest Science*, **68**, 25–32.

Pritsch, K., Courty, P.-E., Churin, J.-L., Cloutier-Hurteau, B., Ali, M.A., Damon, C. *et al.* (2011) Optimized assay and storage conditions for enzyme activity profiling of ectomycorrhizae. *Mycorrhiza*, **21**, 589–600.

R Development Core Team, R. (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Read, D.J., Leake, J.R. & Perez-Moreno, J. (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, **82**, 1243–1263.

Rineau, F., Roth, D., Shah, F., Smits, M., Johansson, T., Canbäck, B. *et al.* (2012) The ectomycorrhizal fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot mechanism involving Fenton chemistry. *Environmental Microbiology*, **14**, 1477–1487.

Rudawska, M., Leski, T. & Stasińska, M. (2011) Species and functional diversity of ectomycorrhizal fungal communities on Scots pine (*Pinus sylvestris* L.) trees on three different sites. *Annals of Forest Science*, **68**, 5–15.

Saikkonen, K., Ahonen-Jonnarth, U., Markkola, A.M., Helander, M., Tuomi, J., Roitto, M. *et al.* (1999) Defoliation and mycorrhizal symbiosis: a functional balance between carbon sources and below-ground sinks. *Ecology Letters*, **2**, 19–26.

Salzer, P. & Hager, A. (1993) Characterization of wall-bound invertase isoforms of Picea abies celts and regulation by ectomycorrhizal fungi. *Physiologia Plantarum*, **88**, 52–59.

Saravesi, K., Markkola, A., Rautio, P., Roitto, M. & Tuomi, J. (2008) Defoliation causes parallel temporal responses in a host tree and its fungal symbionts. *Oecologia*, **156**, 117–123.

Schaeffer, C., Wallenda, T., Guttenberger, M. & Hampp, R. (1995) Acid invertase in mycorrhizal and non-mycorrhizal roots of Norway spruce (Picea abies [L.] Karst.) seedlings. *New Phytologist*, **129**, 417–424.

Smith, S.E. & Read, D. (2008) *Mycorrhizal Symbiosis*, 3rd edn. Academic Press, New York, NY.

Talbot, J.M., Allison, S.D. & Treseder, K.K. (2008) Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Functional Ecology*, **22**, 955–963.

Tedersoo, L., Naadel, T., Bahram, M., Pritsch, K., Buegger, F., Leal, M. *et al.* (2012) Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest. *New Phytologist*, **195**, 832–843.

Treseder, K.K., Balser, T.C., Bradford, M.A., Brodie, E.L., Dubinsky, E.A., Eviner, V.T. *et al.* (2012) Integrating microbial ecology into ecosystem models: challenges and priorities. *Biogeochemistry*, **109**, 7–18.

Wallenstein, M.D., Mcmahon, S.K. & Schimel, J.P. (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Global Change Biology*, **15**, 1631–1639.

Received 4 February 2016; accepted 3 June 2016
Handling Editor: Kathleen Treseder

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Effect of phenology and ectomycorrhizal exploration type on potential enzyme activity.

Table S1. Descriptions of morphotypes included in contact and distance exploration types.

Table S2. Operational taxonomic units of ectomycorrhizal fungi identified using molecular methods.

Appendix S1. Detailed description of enzyme assays and molecular identification of ectomycorrhizal fungi.