Changes in root associated fungal communities during fine root decomposition in *Abies balsamea* and *Picea rubens*

by
Logan Gray

A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfilment of the Requirements for the
Degree of Master of Science in Applied Science

April, 2016, Halifax, Nova Scotia

Approved: Dr. Gavin Kernaghan
Supervisor
Department of Biology, MSVU

Approved: Dr. Zhongmin Dong
Supervisory Committee Member
Department of Biology, SMU

Approved: Dr. Linda Campbell
Supervisory Committee Member
Department of Environmental Science, SMU

Approved: Dr. Allison Walker
External Examiner
Department of Biology, Acadia University

Date: April 26th, 2016

Changes in root-associated fungal communities during fine root decomposition in *Abies balsamea* and *Picea rubens*

by
Logan Gray

Abstract

Fine roots are ephemeral roots < 2 mm in diameter that are frequently replaced during fine root turnover. As fine roots harbour symbiotic and commensal fungi, their trophic strategy may shift upon fine root senescence and decomposition. This study examined fungal communities in *Abies balsamea* and *Picea rubens* fine roots during decomposition. Observations of ectomycorrhizae showed that *Cenococcum geophilum* was recalcitrant, showing no appreciable decomposition after 16 months. Hyaline ectomycorrhizae were least recalcitrant and were not detected beyond four months. Differences between ectomycorrhizal recalcitrance may subsequently affect fine root decomposition. Molecular analysis showed: an ectomycorrhizal community becoming dominated by *Piloderma*; an increase in helotialian endophytes; and a relatively limited presence of saprotrophs. The most common saprotroph detected increased in concert with the endophytes. Increases in endophytic abundance suggests that they may be involved in the decomposition of fine roots in forest soils.

April 26th, 2016

Table of Contents **Page**

Abstract....ii

Table of Contents....iii

List of Figures....vii

List of Tables....ix

Acknowledgements....xi

CHAPTER 1: Introduction....1

References....8

CHAPTER 2: Decomposition of Ectomycorrhizal Fine Roots of *Abies balsamea* and *Picea rubens* over a 16 Month Period in The Acadian Forest....14

Abstract....14

Introduction....14

Materials and Methods....20

Field Sites....20

Seedling Excavation....20

Edaphic Measurements....21

Tea Bag Index....21

Classification of Ectomycorrhizae....23

Statistical Analysis of Mantle Data....24

Measurement of Primary Fine Root Loss....24

Results 25

Edaphic Measurements 25

Primary Fine Root Loss 25

Mantle Integrity 26

Discussion 26

Edaphic measurements 26

Morphological observations of ECM 27

Melanised mantles – Cenococcum geophilum 27

Non-melanised mantles with recalcitrant secondary metabolites – Russulaceae & *Piloderma* 29

Hyaline ECM – Cortinarius, *Hebeloma*, and *Amanita* 31

Conclusion 32

Future Research 33

References 44

CHAPTER 3: Molecular Characterisation of Fine Root Associated Fungal Communities during the Early Stages of Decomposition in the Acadian Forest 60

Abstract 60

Introduction 60

Mycorrhizal Fungi 61

Endophytic Fungi 62

Saprotrophic Fungi 63

Pathogenic Fungi 64

Fine Root Decomposition.....65

Materials and Methods.....66

Sample Collection.....66

DNA Extraction.....66

Polymerase Chain Reaction (PCR).....68

Molecular Cloning.....69

Colony PCR.....69

ITS Sequence Analysis.....70

Sample Diversity, Analysis of Similarity, and Similarity Percentages 70

Results.....71

DNA Sequencing and OTU Assignment.....71

Redundancy Analysis, Analysis of Similarity, and Similarity of Percentages.....71

Ectomycorrhizal Fungi.....72

Endophytic Fungi.....72

Saprotrophic Fungi.....73

Discussion.....73

Ectomycorrhizal fungi.....73

Fungal root endophytes.....77

Saprotrophs.....80

Conclusion.....82

Future Research.....83

References.....96

CHAPTER 4: Research Synthesis and Concluding Remarks 117

References 121

APPENDIX 1: Supplemental Materials for Chapter 2.....122

APPENDIX 2: Supplemental Materials for Chapter 3.....139

List of Figures **Page**

CHAPTER 1: Introduction

Figure 1. Fine roots from *Abies balsamea* with various root orders highlighted.....6
Figure 2. Melanised extraradical hyphae growing from an ectomycorrhiza formed by an unidentified ectomycorrhizal fungus.....7

CHAPTER 2: Decomposition of Ectomycorrhizal Fine Roots of *Abies balsamea* and *Picea rubens* over a 16 Month Period in The Acadian Forest

Figure 1. Location of study sites on mainland Nova Scotia: stars = site location; dark grey = Kejimkujik National Park; white circle = Halifax.....34
Figure 2. Topography of site at Annapolis Road: filled triangles = plots; contours in meters.....35
Figure 3. Topography of site at McGowan Lake: filled triangles = plots; dotted grey = bog; contours in meters.....36
Figure 4. Edaphic measurements by plot: a) soil temperature; b) soil pH; error-bars = 95% confidence intervals.....37
Figure 5. Edaphic measurements by plot: a) soil moisture; b) tea bag index; error-bars = 95% confidence intervals.....38
Figure 6. Linear regression of tea bag index against soil moisture.....39
Figure 7. Primary fine root loss in treatment seedlings of *A. balsamea* and *P. rubens*; error-bars = SE.....40
Figure 8. Mean mantle ranking for mantle categories over 14 months; error bars represent 95% confidence intervals.....41
Figure 9. (a) Melanised ectomycorrhiza of C. geophilum; (b) non-melanised hyphae of an unidentified hyaline ectomycorrhiza.....42

CHAPTER 3: Molecular Characterisation of Fine Root Associated Fungal Communities during the Early Stages of Decomposition in the Acadian Forest

Figure 1. Proportion of OTUs made up by the different functional groups of root associated fungi up to 16 months post-treatment.....86

Figure 2. Ectomycorrhizal community (proportion of OTUs) in pre-treatment and 16 month post-treatment seedlings.....87

Figure 3. Endophytic community (proportion of OTUs) in post-treatment seedlings.....88

Figure 4. Saprotrophic community (proportion of OTUs) in post-treatment seedlings.....89

Figure 5. Redundancy analysis (RDA) for treatment seedlings from both *A. balsamea* and *P. rubens* across all times. Ellipses represent 95% confidence intervals around centroid. Results based on 9,999 permutations.....90

APPENDIX 1: Supplemental Materials for CHAPTER 2

Figure 1. Mean soil temperature for Annapolis Road (AR) and McGowan Lake (ML). Error bars represent 95% confidence interval.....123
Figure 2. Mean soil acidity for Annapolis Road (AR) and McGowan Lake (ML): error-bars represent 95% confidence intervals.....124
Figure 3. Mean soil moisture for Annapolis Road (AR) and McGowan Lake (ML): error-bars represent 95% confidence intervals.....125
Figure 4. 95% family wise confidence levels for pH ~ plot at Annapolis Road (1, 2, 3) and McGowan Lake (4, 5, 6).....126
Figure 5. 95% family wise confidence levels for soil moisture | plot at Annapolis Road (1, 2, 3) and McGowan Lake (4, 5, 6).....127
Figure 6. 95% family wise confidence levels for TBI ~ plot at Annapolis Road (1, 2, 3) and McGowan Lake (4, 5, 6).....128
Figure 7. Primary fine root loss in control and treatment seedlings of *A. balsamea* and *P. rubens* at Annapolis Road (AR) and McGowan Lake (ML): error-bars = SE.....129
Figure 8. Seedling age for *A. balsamea* and *P. rubens* at each site: error-bars represent 95% confidence intervals.....130

APPENDIX 2 – Supplemental Materials for CHAPTER 3

Figure 1. Proportion of the fungal community made up by the most common ectomycorrhizal genera in treatment seedlings.....140

List of Tables **Page**

CHAPTER 2: Decomposition of Ectomycorrhizal Fine Roots of *Abies balsamea* and *Picea rubens* over a 16 Month Period in The Acadian Forest

Table 1. Summary of edaphic measurements for plots (± SE) for plots at Annapolis Road (AR) and McGowan Lake (ML).....43

Table 2. Mantle observations from *A. balsamea* and *P. rubens* seedlings at Annapolis Road (AR) and McGowan Lake (ML).....43

Table 3. Proportional odds calculated using ordinal-logit model.....43

Table 4. Pair-wise comparison of seedling age of *A. balsamea* and *P. rubens* across plots.....43

CHAPTER 3: Molecular Characterisation of Fine Root Associated Fungal Communities during the Early Stages of Decomposition in the Acadian Forest

Table 1. List of literature supporting categorization of OTUs into functional groups.....91

Table 2. OTUs from control and treatment seedlings across all time intervals for *A. balsamea* and *P. rubens*.....93

Table 3. SIMPER scores for OTU similarity between *A. balsamea* and *P. rubens*.....94

Table 4. SIMPER scores for dissimilarity.....95

APPENDIX 1 – Supplemental Materials for CHAPTER 2

Table 1. Edaphic measurements for Annapolis Road Plot 1.....131

Table 2. Edaphic measurements for Annapolis Road Plot 2.....132

Table 3. Edaphic measurements for Annapolis Road Plot 3.....133

Table 4. Edaphic measurements for McGowan Lake Plot 1.....134

Table 5. Edaphic measurements for McGowan Lake Plot 2.....135

Table 6. Edaphic measurements for McGowan Lake Plot 3.....136

Table 7. Initial and final mass of oven dried green and rooibos teas (without bag) after approximately 90 days post burial.....137

Table 8. Descriptions of plots.....138

APPENDIX 2 – Supplemental Materials for CHAPTER 3

Table 1. Collection dates and months post treatment for time intervals. Underlined dates denote time intervals used for metagenomic analysis, dates with asterisks were used for morphological data.....141

Table 2. OTUs assigned to sequences from primary fine roots of *A. balsamea* from Annapolis Road and McGowan Lake. Numbers in parenthesis indicate abundance of the OTU in each sample.....142

Table 3. OTUs assigned to sequences from primary fine roots of *P. rubens* from Annapolis Road and McGowan Lake at various time intervals. Numbers in parenthesis indicate abundance of the OTU in each sample.....143

Table 4. OTUs assigned to sequences from primary fine roots of *A. balsamea* and *P. rubens* from Annapolis Road and McGowan Lake at Time Interval 0 (Controls). Number in parenthesis indicates the abundance of the OTU in each sample.....144

Acknowledgements

First and foremost I'd like to thank Dr. Kernaghan for the time, resources, and effort he put into this research; without his guidance, training, and patience, this would not have been possible. I'm also indebted to Dr. Dong and Dr. Campbell for their constructive comments and input on my project over these past years as well as to Dr. Walker for her expert opinion on my thesis and its contents. I'd like to thank; Michael Mayerhofer, for showing me the ropes at ARSL, sharing his considerable knowledge of statistics and bioinformatics, and our discussions regarding Vaporwave, desert music, and prizza; Laura Johnson and Steph Beland, for letting me hang out with the cool kids; Amanda Griffin, for her assistance in gathering Edaphic measurements from my miasmic sites, her continuous upkeep of laboratory materials, and refreshing curiosity about lichens; Dan Noye, for the use of his 4-wheel drive, time, enthusiasm, and canine side-kick ("Ruby") during visits to my sites during a greasy weekend in November; and finally, Emily Walker, who has put up with my constant ditch-picking, stump-searching, and trespassing to collect fungi on our many hikes and strolls.

CHAPTER 1

Introduction

Although the above-ground sources of plant litter are more apparent, below-ground litter production often matches (Raich & Schlesinger 1992; Xia et al. 2015) and may surpass (Grier et al. 1981) above-ground sources. Below-ground litter is comprised largely of discarded fine roots, which are defined as any roots less than two millimetres in diameter (Goebel et al. 2011; Lukac 2012). Fine roots are excised into soil during fine root turnover, the annual dieback and growth of fine roots in order to maintain optimal water and nutrient absorption (Lukac 2012). Fine root turnover is fastest in the primary fine roots (Goebel et al. 2011) which are often colonised by mutualistic and commensal fungi (Figure 1). Fine root turnover is estimated to account for one third of global terrestrial net primary productivity (Vogt et al. 1986; Jackson et al. 1997) and for 15% of total forest biomass production (Hobbie et al. 2006).

Fine roots comprise a larger portion of soil organic matter (SOM) than other forms of plant litter (McClaugherty et al. 1982; Tisdal & Oades 1982; Koide & Malcolm 2009; Koide 2011; Lukac 2012; Xia et al. 2015). SOM is estimated to represent two to three times as much organic matter than terrestrial vegetation (Koide et al. 2009) and represents more carbon than biotic and atmospheric pools combined (Lal 2004; Davidson & Jannsens 2006). In temperate forests, fine root litter was found to last one-third longer (Harmon et al. 2009; Xia et al. 2015) and retain one-third more carbon (Bird & Torn 2006; Bird et al. 2008) than other forms of litter. The difference in decomposition rate between root and above-ground litter is driven by biochemistry (Taylor et al. 1991;

Abiven et al. 2005; Bird et al. 2008; Xia et al. 2015). The acid-insoluble-fraction (*e.g.* lignin, tannins, suberins) of fine root litter and leaf litter was found to be 45% and 15%, respectively, with the tannin content at 14% and 6%, respectfully. When fine root litter enters the soil, early saprotrophic communities digest labile compounds, leaving the recalcitrant compounds for successional groups (Eklund & Gyllenberg 1974; Boddy & Watkinson 1994; Torres 2005). The speed at which these recalcitrant compounds degrade limits how long carbon is sequestered in these soils.

Many fungi have the enzymatic ability to decompose plant cell wall components, a property that makes them essential in decomposition. Fungi release digestive enzymes externally which depolymerise organic macromolecules, resulting in monomeric compounds that are easily absorbed and used in various metabolic processes (Sinsabaugh 1992). However, extracellular digestion is inefficient; some of the monomers "leak" into the soil environment and become utilised by other organisms. The ability to liberate sequestered nutrients makes fungi (and other saprotrophs) integral components of nutrient and carbon cycling (Bruns et al. 2013).

Specialized enzyme production represents a high energy cost, so many saprotrophs specialise in digesting particular substrates (Hanson et al. 2008; McGuire et al. 2010). Therefore, decomposition can be understood as a property of the saprotrophic community and the enzymes they excrete (Marsden & Gray 1986; Saddler 1986; Kirk & Farrell 1987). Although many saprotrophic organisms are capable of digesting the structural carbohydrates of the plant cell wall, fungi are unique as they are able to entirely decompose lignin (de Boer et al. 2005; Hattenschwiller et al. 2005), a recalcitrant

structural compound made of non-repeating, phenolic rings (Kirk & Farrell 1987). In the plant cell wall, cellulose microfibrils are interspersed with lignin, which acts as a physical barrier to microbes without the ability to digest lignin (Blanchette 1991). Fungi create multicellular networks of hyphae (mycelium) that infiltrate lignin-containing substrates through the secretion of non-specific oxidases. These extracellular enzymes unbind and modify lignin (Blanchette 1991; de Boer et al. 2005) and degrade the substrate into smaller portions. Such substrate transformation exposes more surface area to bacteria and other saprotrophs which can begin to digest the structural carbohydrates.

Not all fungi are saprotrophic; many fungi enter symbiotic relationships with plants through their primary fine roots. For example, ectomycorrhizal (ECM) fungi are symbionts that have evolved mutualistic partnerships with the fine roots of plants, especially trees (Fogel & Hunt 1983). Fine root and fungal tissues combine at the cellular level to create the mycorrhiza, a hybrid structure which acts as the interface for metabolic exchange between the two organisms (Smith & Read 2008). Mycorrhizal relationships are a crucial component in optimal tree growth with as much as 30% of photosynthate being translocated below-ground into root-associated fungal biomass (Hobbie 2006). The extramatrical hyphae of ECM fungi (Figure 2) have a high surface area to volume ratio, granting them access to soil nutrients and water that is unachievable by root hairs. In return for increased access to these resources, trees translocate sugars to the ECM fungi. Some fungi live commensally in root tissues without apparent detriment to plant health (Schulz & Boyle 2005, 2006; Mayerhofer et al. 2013). These fungal endophytes inhabit much of the root system but are concentrated in the fine roots (Jumpponen & Trappe

1998; Grünig et al. 2008; Wagg et al. 2008). These cryptic fungi are difficult to identify and modern molecular techniques will hopefully shed more light on their natural history. As expected, relationships that benefit fungi at the expense of plant health also exist. Pathogenic fungi use specialized hyphae to penetrate the root epidermis (Perfect & Green 2001), colonising the root cortex and hydrolyzing plant cells for nutrition (Raajimakers et al. 2009).

Although these categories are useful conceptually, they are not discrete and could be better understood as occurring on a biotrophic-saprotrophic continuum (Shultz & Boyle 2005), as some fungi may be triggered by environmental cues to become more saprotrophic (Shultz & Boyle 2006). Little is understood regarding how mutualistic and commensal fungi respond to tissue senescence; if these fungi have the ability to change trophic strategy, the residence time of fine root litter (and SOM) in forest soils could be affected. If so, a biotic factor such as fungal succession could be integrated into decomposition models which contain mostly abiotic factors (Kirschbaum 1995; Hendrick & Pregitzer 1997; Solly et al. 2014).

There are contrasting reports in the literature as to whether ECM colonisation increases or decreases the rate of fine root decomposition (Langley & Hungate 2003; Langley et al. 2006; Koide et al. 2011). This is unsurprising as the recalcitrance of ECM (Koide & Malcolm 2009; Koide et al. 2011) and their enzymatic capabilities can vary significantly (see Chapter 3). These differences may influence the decomposition rate of fine root litter. The role of endophytic fungi in fine root decomposition is even less understood. The vague definition of what an endophyte is contributes to this confusion

(Chapter 3). Additionally, the cryptic nature of many endophytes made them difficult to study prior to modern molecular techniques and research into their ecological function has started only relatively recently.

This study was designed to analyse how fungal communities respond to the senescence of fine root tissue. The fine root systems of *Abies balsamea* (balsam fir) and *Picea rubens* (red spruce) were chosen for study as these species comprise large portions of coniferous dominated forests in the maritime provinces of Canada. Seedlings of both species were killed and their fine root systems were analysed over a 16 month period. The second chapter of this thesis examines the relative recalcitrance of some common ECM genera as they decompose as well as determining how quickly primary fine roots are lost from root systems during fine root decomposition. The third chapter describes the molecular results of fungal community succession during fine root senescence and decomposition. In the fourth and final chapter, the observations from the previous chapters are synthesised to draw conclusions regarding how the fungal response to fine root senescence may influence decomposition in forest soils.

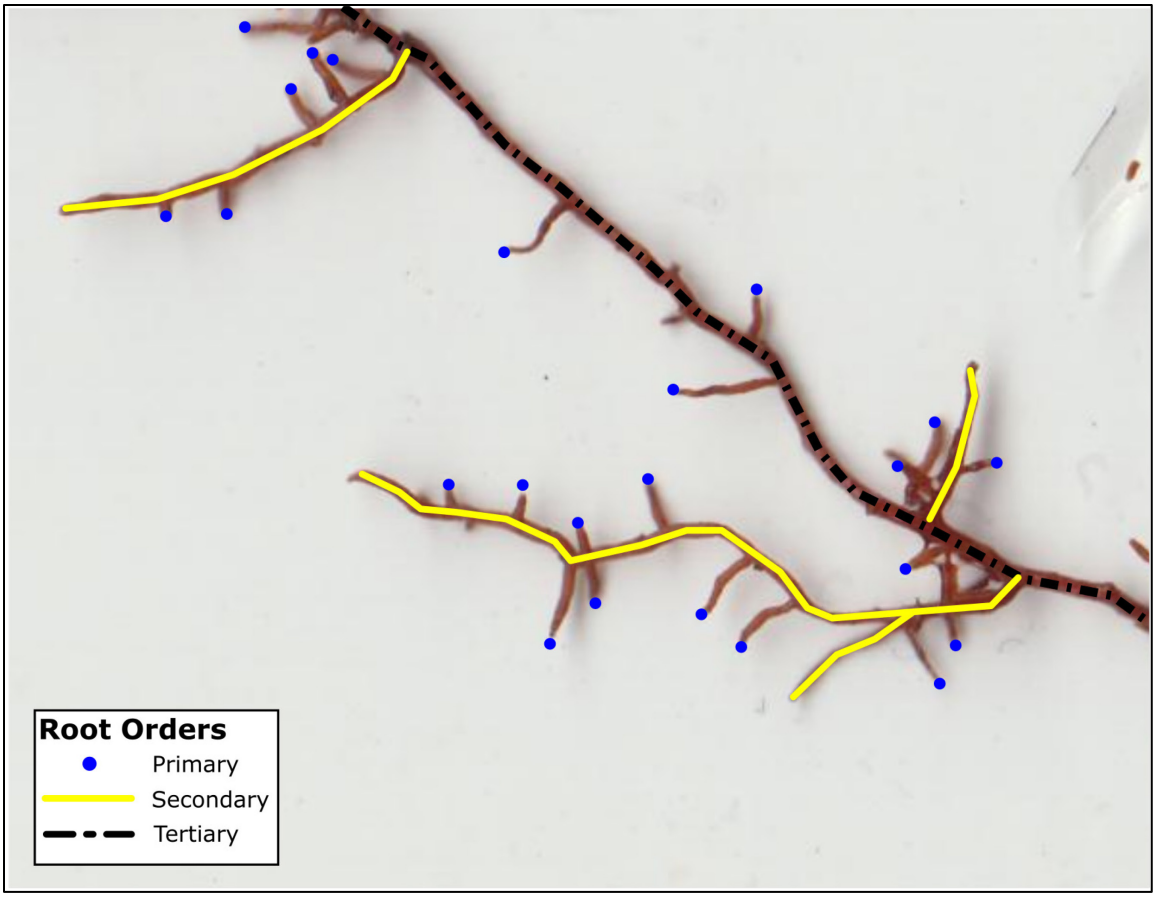

Figure 1. Fine roots from *Abies balsamea* with various root orders highlighted.

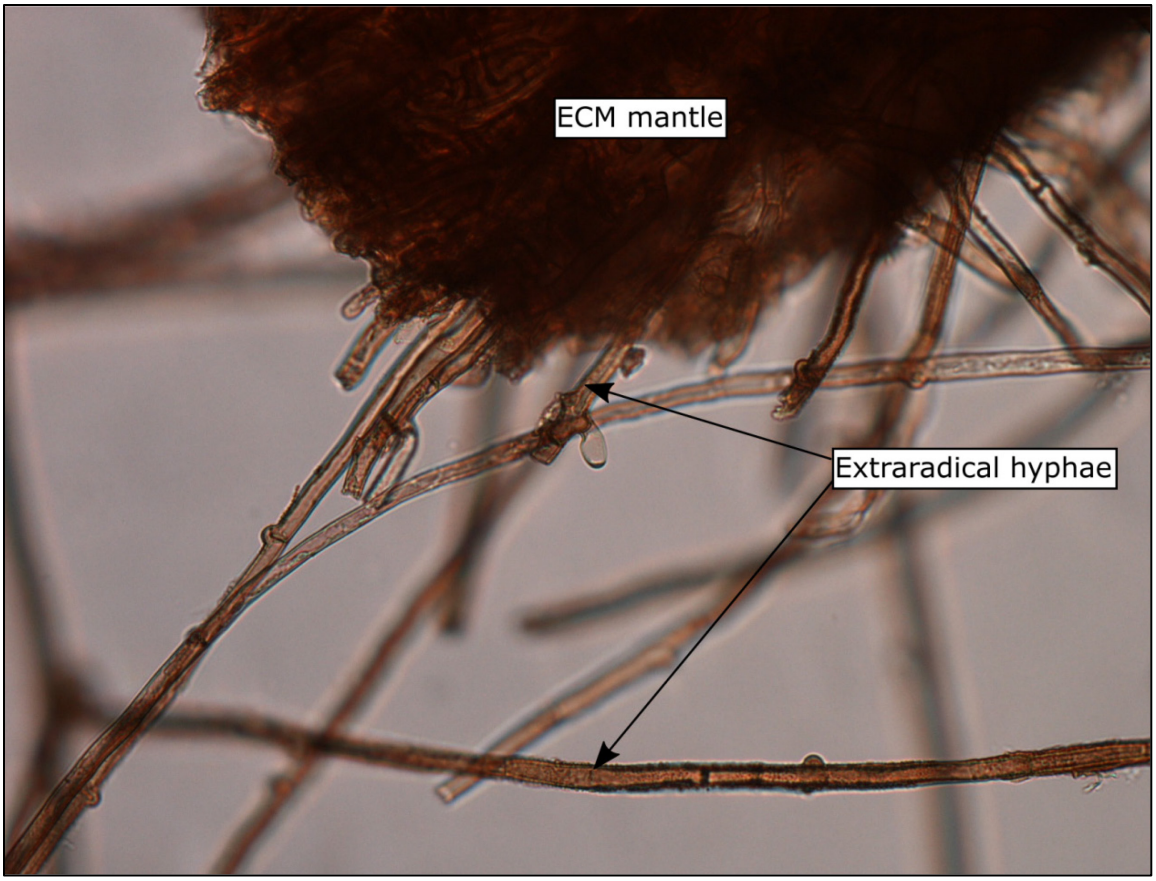

Figure 2. Melanised extraradical hyphae growing from an ectomycorrhiza formed by an unidentified ectomycorrhizal fungus.

References

Aber, J. D., Melillo, J. M., Nadelhoffer, K. J., McClaugherty, C. A., & Pastor, J. (1985). Fine root turnover in forest ecosystem in relation to quantity and form of nitrogen availability: a comparison of two methods. *Oecologia* **66**:317-321.

Abiven, S., Recous, S., Reyes, V., & Oliver, R. (2005). Mineralisation of C and N from root, stem and leaf residues in soil and role of their biochemical quality. *Biology and Fertility of Soils* **42**:119-128.

Bird, J. A., Kleber, M., & Torn, M. S. (2008). 13 C and 15 N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. *Organic Geochemistry* **39**:465-477.

de Boer, W., Folman, L. B., Summerbell, R. C., & Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* **29**:795-811.

Caldwell, M. M. & Richards, J. H. (1986). *On the economy of plant form and function.* University Press: Cambridge.

Davidson, E. A. & Jannsens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature Reviews* **440**:165-173.

Dixon, R. K., Brown, S., Houghton, R. A., Solomon, A. M., Trexler, M. C., & Wisniewski, J. (1994). Carbon pools and flux of global forest ecosystems. *Science* **263**:185-190.

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

Goebel, M., Hobbie, S. E., Bulaj, B., Zadworny, M., Archibald, D. D., Oleksyn, J., Reich, P. B., & Eissenstat, D. M. (2011). Decomposition of the finest root branching orders: linking belowground dynamics to fine-root function and structure. *Ecological Monographs* **81**:89-102.

Grier, C. C., Vogt, K. A., Keyes, M. R., & Edmonds, R. L. (1981). Biomass distribution and above-and below-ground production in young and mature *Abies amabilis* zone ecosystems of the Washington Cascades. *Canadian Journal of Forest Research* **11**:155-167.

Grünig, C. R., Queloz, V., Sieber, T. N., & Holenrieder, O. (2008). Dark septate endophytes (DSE) of the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex in tree roots: classification, population biology and ecology. *Botany* **86**:1335-1369.

Heimann, M. & Reichstein, M. (2008). Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**:289-292.

Hendrick, R. L. & Pregitzer, K. S. (1992). The demography of fine roots in a northern Deciduous forest. *Ecology* **73**:1094-1104.

Hobbie, E. A. (2006). Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology* **87**:563-569.

Jackson, J. B., Mooney, H. A., & Schulze, E.D. (1997). A global budget for fine root biomass, surface area, and nutrient contents. *Proc. Natl. Acad. Sci.* **94**:7362-7366.

Jumpponen, A. & Trappe, J. M. (1998). Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytol.* **140**:295-310.

Kernaghan, G. (2013). Functional diversity and resource partitioning in fungi associated with the fine feeder roots of forest trees. *Symbiosis* **61**:113-123.

Kirschbaum, M. U. F. (1995). The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biol. Biochem.* **27**:753-760.

Koide, R. T. & Malcolm, G. M. (2009). N concentration controls decomposition rates of different strains of ectomycorrhizal fungi. *Fungal Ecology* **2**:197-202.

Koide, R. T., Fernandez, C. W., & Peoples, M. S. (2011). Can ectomycorrhizal colonization of *Pinus resinosa* roots affect their decomposition? *New Phytol.* **191**:508-514.

Koide, R. T., Shumway, D. L., Xu, B., & Sharda, J. N. (2007). On temporal partitioning of a community of ectomycorrhizal fungi. *New Phytol.* **174**:420-429.

Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science* **304**:1623-1627.

Lindahl, B. & Tunlid, A. (2015). Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytol.* **205**:1443-1447.

Lukac, M. (2012). *Measuring Roots: An Updated Approch.* Springer-Verlag: Berlin-Heidelberg.

Mayerhofer, M. S., Kernaghan, G., & Harper, K. A. (2013). The effects of fungal root endophytes on plant growth: a meta-analysis. *Mycorrhiza* **23**:119-128.

McClaugherty, C. A., Aber, J. D., & Melillo, J. M. (1982). The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. *Ecology* **63**:1481-1490.

Nadelhoffer, K. J. & Raich, J. W. (1992). Fine root production estimates and belowground carbon allocation in forest ecosystems. *Ecology* **73**:1139-1147.

Perfect, S. E. & Green, J. R. (2001). Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. *Molecular Plant Pathology* **2**:101-108.

Raajimakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil* **321**:341-361.

Raich, J. W., & Schlesinger, W. H. (1992). The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus B* **44**:81-99.

Schulz, B. & Boyle, C. (2005). The endophytic continuum. *Mycol. Res.* **109**:661-686.

Schulz, B. & Boyle, C. (2006). *What are endophytes? Soil Biology Volume 9: Microbial Root Endophytes*. Springer-Verlag: Berlin-Heidelberg.

Smith, S. E., & Read, D. (2008). *Mycorrhizal Symbiosis (Third Edition)*. Academic Press: Cambridge.

Solly, E. F., Schöning, I., Boch, S., Kandeler, E., Marhan, S., Michalzik, B., Müller, J., Zscheischler, J., Trumbore, S. E., & Schrumpf, M. (2014). Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. *Plant Soil* **383**:203-218.

Sun, T., Mao, Z., & Han, Y. (2013). Slow decomposition of very fine roots and some factors controlling the process: a 4-year experiment in four temperate tree species. *Plant Soil* **372**:445-458.

Swift, J. M., Heal, O. W., & Anderson, J. M. (1979). *Decomposition in terrestrial ecosystems.* University of California Press: Oakland.

Taylor, B. R., Prescott, C. E., Parsons, W. J. F., & Parkinson, D. (1991). Substrate control of litter decomposition in four Rocky Mountain coniferous forests. *Canadian Journal of Botany* **69**:2242-2250.

Tisdal, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. *Journal of Soil Science* **33**:141-163.

Vogt, K. A., Grier, C. C., & Vogt, D. J. (1986). *Production, turnover, and nutrient dynamics of above and belowground detritus of world forests*. Academic Press: Cambridge.

Vogt, K. A., Vogt, D. J., Palmiotto, P. A., Boon, P., O'Hara, J., & Asbjornsen, H. (1996). Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant and Soil* **187**:159-219.

Wagg, C., Pautler, M., Massicotte, H. B., & Peterson, R. L. (2008). The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae. *Mycorrhiza* **18**:103-110.

Xia, M., Talhelm, A. F., & Pregitzer, K. S. (2015). Fine roots are the dominant source of recalcitrant plant litter in sugar maple-dominated northern Deciduous forests. *New Phytol.* **208**:715-726.

CHAPTER 2

Decomposition of Ectomycorrhizal Fine Roots of *Abies balsamea* and *Picea rubens* over a 16 Month Period in The Acadian Forest

Abstract

Fine roots are one of the most important components of carbon and nitrogen cycling in temperate forests. Senescent fine root tissues are continuously replaced with new growth during the process of fine root turnover. Fine roots are also the site of various types of symbiotic relationships with fungi. In particular, the fine roots of coniferous trees have well developed relationships with ectomycorrhizal fungi. This study focuses on how several common ectomycorrhizae of *Abies balsamea* and *Picea rubens* decompose after fine root senescence. Ectomycorrhizal fungi were categorized based on mantle characteristics. The highly melanised mantles of *Cenococcum geophilum* decomposed little over the 16 month study period, while non-melanised mantles with the presence of antifeedant or recalcitrant compounds showed intermediate rates of decomposition. Finally, mantles composed of hyaline hyphae decomposed most rapidly and were not observed beyond 12 months post treatment.

Introduction

Fine roots are the functional interface between tree and soil where water and nutrients are absorbed (Lukac 2011). In general, fine roots are ephemeral roots of trees, typically ≤ 2mm in diameter (Finer et al. 2011; Goebel et al. 2011). Because of their highly important function, trees continuously allocate resources to maintain their fine roots. When fine roots become senescent, they abscise, are released into the rhizosphere, and replaced by new fine roots (Finér et al. 2011; Lukac 2012) in the process of fine root turnover. Specifically, fine root turnover is defined as the ratio of fine root production to standing crop of fine roots annually (Dahlman & Kucera 1965; Gill & Jackson 2000). A more general definition of fine root turnover describes it as the entire process of fine root production, senescence, and decomposition (Joslin et al. 2000) and is the definition used in this study. Fine root turnover shows both intra- and inter-specific variation in addition

to being influenced by a variety of environmental factors (Keyes & Grier 1981; Vogt et al. 1983; Vogt et al. 1987; Finér et al. 1997; Finér & Laine 1998; Joslin et al. 2000; Pregitzer et al. 2000; Lahti et al. 2005; Tingey et al. 2005; Hendricks et al. 2006; Cusack et al. 2009; Prescott 2010; Fujii & Takeda 2010; Aulen et al. 2012)

Conservative estimates report fine root turnover accounting for one third of annual terrestrial net primary productivity (NPP) (Vogt et al. 1986; Jackson et al. 1997; Hobbie 2006; McCormack et al. 2015) with more liberal estimates reaching 75% NPP (Keyes & Grier 1981; Vogt et al. 1996; Gill & Jackson 2000). The technological difficulties involved in measuring fine root turnover has made accurate measurements difficult (Hendricks et al. 2006; Finér et al. 2011). Regardless of these discrepancies, fine root turnover is a fundamental component of carbon and nutrient cycling in terrestrial ecosystems (Finér et al. 2011), profoundly affecting soil ecology (Tisdal & Oades 1982; McClaugherty et al. 1982; Koide & Malcolm 2009; Koide 2011; Lukac 2012) by altering carbon and nitrogen availability in forests (Aber et al. 1985).

During fine root turnover, large amounts of fine root detritus enters the forest floor. This fine root litter decomposes more slowly than above-ground litter (Fan & Guo 2010; Fujii & Takeda 2010; Sun et al. 2013; Xiong et al. 2013) and is a major component of SOM (Fujii & Takeda 2010; Kätterer et al. 2011; Xiong et al. 2013) and may sequester more carbon than biotic and atmospheric pools combined (Batjes 1996; Lal 2004; Davidson & Jannsens 2006; Schmidt et al. 2011). Understanding the dynamics of fine root decomposition is crucial to developing better carbon models as litter decomposition rates strongly affect how much carbon is sequestered in forest soils (Lal 2004).

Regarding chemical composition, Sun, Mao, and Han (2013) categorise fine root constituents into three main categories: 1) labile compounds such as fats, oils, waxes, polyphenols, and non-structural carbohydrates; 2) structural compounds that are hydrolysable in acid such as cellulose and hemicellulose; and 3) structural compounds that are not hydrolysable in acid such as lignin, suberin, cutin, and tannins. The authors state that the concentration of unhydrolysable structural carbon (*i.e.* lignin) dictates fine root decomposition rates (Sun et al. 2013) which have high ratios of carbon to nitrogen (C:N). Studies of above-ground litter decomposition demonstrate that tissues with high C:N ratio (meaning low N content) decompose slowly relative to compounds with high N content (Melillo et al. 1982; Taylor et al. 1989; Fenn 1991; Cotrufo et al. 1994; Aerts 1997; Berg 2000). A meta-analysis of fine root decomposition studies found that C:N ratio explained approximately 85% of variation in fine root decomposition rates (Silver & Miya 2001) , agreeing with several other studies (Berg 1984; Ostertag & Hobbie 1999; Trofymow et al. 2002; Lin et al. 2011). Labile compounds that are easily utilised by microorganisms (monomeric or dimeric sugars) are also strong indicators of fine root decomposition rate (Chen et al. 2002; Fan & Guo 2010; Hobbie et al. 2010; Aulen et al. 2012; Birouste et al. 2012). Manganese content, root pigmentation, and tissue density have also been suggested as playing a role in fine root decomposition (Hobbie et al. 2010; Goebel et al. 2011; Aulen et al. 2012; Birouste et al. 2012). Considering the lack of consensus, more research is necessary to better understand which factors most strongly affect fine root decomposition rate.

Ectomycorrhizal (ECM) colonization of fine roots is a potential factor regulating fine root decomposition that has only begun to be described. During the colonization process, the eponymous ectomycorrhiza is created; this hybrid structure of fine root and fungal tissues varies chemically and morphologically between ECM species. In temperate and boreal forests, ecologically dominant Pinaceae (Koide et al. 2011) are highly colonized by ECM fungi (Allen et al. 1995; Dahlberg 2001) where ECM may account for a third of microbial biomass (Fogel & Hunt 1983; Högberg & Högberg 2002) and up to 84% of fungal biomass in soils (Bååth et al. 2004). Coniferous forests may allocate up to 22% of their carbon to ectomycorrhizal symbionts (Hobbie 2006). As ECM fungi produce a layer of fungal tissue (mantle) ensheathing the fine root, it is logical that these fungi may affect fine root decomposition (Langley et al. 2006; Fernandez & Koide 2011; Koide et al. 2011). Any factors that control decomposition of ECM fungi may also affect decomposition of fine root litter, consequently influencing C and nutrient cycling in forests (Koide et al. 2011).

The few studies regarding the ectomycorrhizal effect on fine root decomposition give contradictory findings regarding whether fungal colonization increases or decreases decomposition rate. Langley, Chapman, and Hungate (2006) found that ECM colonization significantly decreased the decomposition rate of fine roots in a *Pinus edulis* plantation. In contrast, Koide, Fernandez, and Peoples (2011) found no significant difference in decomposition rate between mycorrhizal and non-mycorrhizal fine roots of *Pinus edulis*. These contrasts are likely caused by the multiple differences in environmental and biological factors that likely affect fine root decomposition. As studies

on this subject are few (Koide & Malcolm 2009) and as those that exist are contradictory, a better characterization of factors driving ECM decomposition is necessary.

Fungal tissue differs greatly from plant tissue. Fungal cell walls are comprised of glucans, glycoproteins, and chitin. Glucans are glucose polymers differentiated by their type of glycosidic bond. The main structural component of the fungal cell wall is β-1,3-glucan (Bowman & Free 2006), making up 50 – 60% by dry weight (Fleet 1991; Kapetyn et al. 1999). Glycoproteins are the next most abundant component of the fungal cell wall making up 15 – 50% of fungal tissue by dry weight (Fleet 1991; Brown & Catley 1992; Bowman et al. 2006). The final component, chitin (β-1,4-linked N-acetylglucosamine) is a structural component (Bowman et al. 2006), making up only 1 – 2% of the fungal cell wall by dry weight (Klis 1994; Klis et al. 2002).

There is a general consensus that chitin is resistant to decomposition; however there is little evidence to support this claim (Fernandez & Koide 2011) and some studies have found that chitin is less recalcitrant than cellulose (Okafor 1966; Trofymow et al. 1983; Fernandez & Koide 2011). Therefore, it is unlikely that differences in chitin concentration between ECM fungi would drive decomposition rate. Similarly, the glucan and glycoprotein components of fungal cell walls are unlikely to inhibit decomposition as both are N rich and thus likely targeted early by saprotrophic microbes.

Some studies show that pigmented secondary metabolites like melanins greatly increase recalcitrance of fungal tissues (Paris et al. 1993; Butler & Day 1998a). The highly melanised ECM fungus *Cenococcum geophilum* has been found to take

significantly longer to decompose than unmelanised ectomycorrhizal species (Koide & Malcolm 2009; Fernandez et al. 2013). The inclusion or exclusion of highly melanised fungi such as *C. geophilum* in an analysis may explain why these few studies contrast one another. In the study by Koide et al. (2011), the authors examined fungal genera that were not melanised (*e.g. Amanita, Lactarius, Tylopilus, Russula,* and *Suillus*) whereas the study by Langley et al. (2006) (which came to the opposite conclusion) was dominated by *Geopora,* a tomentelloid fungus known for dark, melanised pigmentation (Hrynkiewicz et al. 2015). Nevertheless, pigmented metabolites are probably not the only factor regulating decomposition as some non-melanised fungi have also shown considerable recalcitrance (Koide & Malcolm 2009). Some secondary metabolites that have antimicrobial or antifeedant properties likely increase their recalcitrance (Krywolap 1964; Mandyam & Jumpponen 2005; Rohlfs et al. 2007).

As both fungal and fine root tissues have innate biochemical traits, fine root decomposition may be highly dependent on both. When considering C and nutrient cycling in forest soils, plant *and* fungal tissues need to be considered (Koide & Malcolm 2009). Due to the lack of knowledge regarding this subject, one of the goals of this study was to analyse how some common ECM species of *Abies balsamea* and *Picea rubens* differ in their rate of decomposition.

Materials and Methods

Field Sites

Two sites in the Acadian Forest were selected for study (Figure 1), one near McGowan Lake, NS (44°26'12"N, 65°3'51"W) (Figure 2) and another near Annapolis Road, NS (44°45'6"N, 63°56'36"W) (Figure 3). Sites were located in protected natural areas to prevent accidental human disturbance. Each site contained three 10 m^2 plots in areas with extensive seedling regeneration. Selected plots required a minimum number of seedlings of each species and did not vary dramatically in seedling size (Figure 10). Descriptions of canopy cover and dominant trees are provided (Appendix 1; Table 8).

Each plot was separated into a 4 x 4 grid and numbers from 1 to 16 (corresponding to grid position) were randomly generated. The seedling closest to the centre of each determined by each random number was then randomly assigned to a group (treatment or control) and time interval (zero through 12). Seedlings in the treatment group were severed using secateurs just above the root collar, while seedlings in the control group were not disturbed. Information on site, group, species, plot, and time interval was inscribed on aluminium tags and attached to seedlings with care not to inhibit growth.

Seedling Excavation

Treatment began on June 21st, 2013 with subsequent monthly harvests occurring until October 21st, 2014. Sampling was halted for four months during winter from November 2013 to April 2014. Upon excavation, a soils knife was used to cut seedlings

out of the forest floor. Excavated seedlings were placed in polyethylene bags and transported on icepacks until storage in a cold room at 4°C.

Edaphic Measurements

Soil moisture, temperature, and pH for each plot was measured monthly using a Kelway Soil pH and Moisture Reader (KTL Instruments Company Inc.) and a digital thermometer. Three measurements for each parameter were taken from randomized locations at each plot using the same grid system used for seedling selection. Soil samples taken in October 2014 were analysed for potassium-chloride extractable nitrate (NO_3-N) and ammonium (NH_4-N) levels by the Agriculture & Food Laboratory at the University of Guelph (Guelph, ON, Canada).

Tea Bag Index

Baseline decomposition values for each plot were calculated according to the methods listed in Keuskamp et al. (2013). By calculating mass loss in green and rooibos tea over a 90 day period, a baseline decomposition rate was calculated for each plot. Rooibos and green tea have different concentrations of acid hydrolysable compounds. This difference in decomposability allows for the calculation of decomposition rate without a time series. Tetrahedron nylon tea bags (mesh size 0.25 mm) containing either rooibos or green tea (Lipton, Unilever) were buried pair-wise at each plot in triplicate. Tea bags were oven dried at 60°C for 24 hours, weighed (PI-214, Denver Instrument, NY), and then buried in the forest floor on June 21st, 2014. Variance in burial depth (3 to 7 cm) resulted from different soil depths at plots.

After 90 days, tea bags were unearthed, cleaned of debris, dried at 60°C in an oven for 24 hours, and reweighed. Mass loss for each tea type was used to generate a decomposition rate for each plot:

$$W(t) = ae^{-k_1 t} + (1-a)e^{-k_2 t} \qquad \text{[equation 1]}$$

where $W(t)$ is the mass of the substrate after t = 90 days, a = the labile portion of litter, 1 – a = the recalcitrant portion of the litter, and k_1 and k_2 are the rate constants for labile and recalcitrant fractions, respectively. As the recalcitrant portion of the litter is broken down slowly and would only be measureable over long periods of time, the overall decomposition rate k is mostly determined by k_1. If one assumes that k_2 essentially equals zero, equation 1 can be simplified:

$$W(t) = ae^{-kt} + (1-a) \qquad \text{[equation 2]}$$

where a becomes the labile portion, which is essentially equal to the limit value – the maximum amount of loss that can occur (Berg & Meentemeyer 2002). As the decomposition rate of green tea is higher than that of rooibos, the labile portion in green tea is consumed before the labile portion in rooibos. Using this difference, the decomposable fraction of green tea and the decomposition rate k can be calculated using a single time period. To solve for k in the simplified equation (equation 2), the decomposable portion of rooibos (a_r) must be estimated. To do this, the decomposable portion as measured in the field (a) is used with the chemically expected decomposable portion (H), given by Keuskamp et al. (2013). The deviation between H and a can be used to interpret the inhibiting effect that the environmental conditions had on the

decomposition of the labile fractions in each tea, which is assumed to be equal for both types of tea:

$$S = 1 - \frac{a_g}{H_g} \qquad \text{[equation 3]}$$

where S is the stabilisation factor, a_g is the observed portion of green tea that decomposed, and H_g is the chemically expected decomposable portion. The observed decomposable portion of rooibos (a_r) is estimated multiplying the chemically calculated decomposable fraction of rooibos (H_r) by the stabilisation factor:

$$a_r = H_r(1 - S) \qquad \text{[equation 4]}$$

To summarize, observed decomposition in green tea (a_g) is compared to the chemically calculated decomposable portion of green tea (H_g) to derive the stabilisation factor *S.* This factor, multiplied by the chemically calculated decomposable portion of rooibos (H_r), gives an estimate of the observed decomposition of rooibos (a_r). With *W* and *a* known for both tea types, decomposition rate *k* can be calculated using the simplified equation (equation 2).

Classification of Ectomycorrhizae

Primary fine roots were sampled from untreated seedlings as well as seedlings at two, four, 11, and 14 months post treatment. These times were chosen as they represent a chronology of the study duration but also because fine roots from other time intervals were used for genetic analysis (see Chapter 3). Up to 100 primary fine roots (depending on the number available) from each seedling were cut into 1 cm long pieces and placed in a photographic developer tray inscribed with a 20 piece grid. Distilled water was added to

the tray until root fragments were evenly distributed. A random number generator (https://www.random.org) was used to generate random numbers from 1 to 20 correlating a grid positions. The primary fine root closest to the centre of each grid position was then chosen for observation. The state of the fungal mantle was ranked on a scale of 1 to 4 based on the percentage of mantle present: 1 = < 33%, 2 = 33 - 66%, 3 = 66 - 99%, and 4 = ~ 100%. Ectomycorrhizae were identified to genera using the Colour Atlas of Ectomycorrhizae (Agerer 1987) and the online ectomycorrhizal database Deemy (www.deemy.de). Ectomycorrhizal identifications were also corroborated by the DNA sequencing conducted for the work in Chapter 3. Data on all ECM were then placed into four categories for analysis: 1) *Cenococcum*; 2) Russulaceae; 3) *Piloderma*; and 4) Hyaline ECM (*e.g. Amanita, Cortinarius,* and *Inocybe*).

Statistical Analysis of Mantle Data

An ordered logit model was used to compare differences in mantle integrity scores between the different classes of ectomycorrhizae. A model was fit for mantle rank as a product of ECM category and site using the "ordinal" package (Christensen 2015) in R 3.1.3 (R Foundation for Statistical Computing, Vienna, Austria).

Measurement of Primary Fine Root Loss

Soil and debris were removed from root systems by running under cold water. The three longest secondary fine roots from each seedling were scanned at 600 dpi (CanoScan LiDE 700F, Canon Inc.). Scanned images were used to determine secondary fine root length (cm) and to count the total number of primary fine roots (NIS Elements BR 2.20, Build 239, Nikon Inc.).

Results

Edaphic Measurements

Mean soil temperature was not significantly different between plots with average soil temperatures ranging from 10.4 to 11.1°C (Figure 4; Table 1). Soil pH varied most at Annapolis Road; the average pH of plot 1 was 5.2 (± 0.1) which was significantly lower than plot 3 and all plots at McGowan Lake which were closer to 5.7 (± 0.1) (one-way ANOVA; $F = 5.6$, $p << 0.001$) (Figure 5; Table 1). Plot 2 at Annapolis Road showed an intermediate pH of 5.5 (± 0.1) (Table 1). Soil moisture was significantly different between plots (one-way ANOVA; $F = 9.7$, $p << 0.001$) with plots 1 (69.2 ± 3.4%) and 3 (71 ± 3.9%) at Annapolis Road being significantly higher than all plots at McGowan Lake (ranging from 46.1 to 53.3 %) with plot 2 showing intermediate levels (Figure 6; Table 1). Decomposition rate as calculated by the Tea Bag Index (TBI) was significantly different between plots (one-way ANOVA; $F = 4.9$; $p = 0.013$) being highest at McGowan Lake plot 1 (0.009 ± 0.001) (Figure 7; Table 1). TBI showed a significant negative correlation with soil moisture ($F = 13.8$, $p = 0.002$, adj. $R^2 = 0.445$ (Figure 8). Edaphic measurements over the course of the study are available in the supplemental materials (Appendix 1).

Primary Fine Root Loss

The ratio of primary fine roots per length of secondary fine root was stable or showed a minor increase in control seedlings (Figure 9). Treatment seedlings of *P. rubens* showed a significant loss of primary fine roots (one-way ANOVA; $F = 19.9$, $p = 0.005$, adj. $R^2 = 0.52$) whereas *A. balsamea* did not (Figure 9). Primary fine root loss did

not show significant correlations between any edaphic factors. Plot and seedling species had a significant interaction (two-way ANOVA; $F = 5.8$, $p << 0.001$) with an interaction between tree species and age ($p = 0.003$) (Figure 10; Table 2).

Mantle Integrity

Across all sampling times, the majority of mantles belonged to *C. geophilum* with the next most abundant belonging to the Russulaceae; *Piloderma* and hyaline hyphae were the least abundant, and made up approximately similar percentages of samples (Table 3). Using an ordinal model with a logit link, ECM category and site were significant predictors of mantle degradation *i.e.* assigned mantle rank (Table 4). All statistical tests for this study used packages developed by the R Core Team (2015) and were completed in R 3.2.3 – "Wooden Christmas-Tree" (R Foundation for Statistical Computing, Vienna, Austria).

Discussion

Edaphic measurements

The variation in the soil properties of plots was expected as plots were located in considerably different environments (e.g. bog edge vs. deep forest). Annapolis Road plot 3 and McGowan Road plot 3 were both located near bog and showed the lowest decomposition rates. Plot 1 at Annapolis Road was located approximately 100 meters down slope from an access road (Figure 2) which may be why soils here were significantly more acidic and more saturated. The high decomposition rate on plot 1 at McGowan Lake is likely a product its lower soil moisture, higher pH, and relatively high proportion of deciduous trees (Appendix 1; Table 8).

Morphological observations of ECM

After measuring ECM decomposition over a 16 month period, the amount of mantle degradation appeared to be dependent on mantle category. The highly melanised *C. geophilum* mantles were relatively unchanged 16 months post treatment, while mantles in the other categories showed significant degradation. A cumulative link mixed model was chosen for the mantle data as the dependent variable (mantle integrity) was ordinal and independent variables consisted of both fixed (treatment group and site) and random (time interval) effects. In a similar experiment by Fernandez et al. (2013) where ECM viability was scored ordinally, a cumulative link mixed model was also used. Based on the model, mantles degraded more quickly at McGowan Lake compared to Annapolis Road. This is intuitive as Annapolis Road had plots with higher acidity and moisture than McGowan Lake. McGowan Lake also contained the plot with the highest decomposition value observed. These observations suggest that ECM species and edaphic factors may have an effect on ECM decomposition in forest soils.

Melanised mantles – Cenococcum geophilum

The recalcitrance observed in *C. geophilum* may be a result of its high concentrations of melanin (Figure 12a). Similar to lignin, melanins are complex biopolymers comprised of phenolic and indolic rings (Butler & Day 1998a; Fernandez & Koide 2014; Fernandez et al. 2016) and lack the stereo-specific binding sites used by hydrolytic enzymes (Butler & Day 1998b). This property requires the production of oxidative enzymes by microbes in order for melanins to be degraded (Fernandez et al. 2016). In addition to inhibiting enzymatic degradation, highly melanised fungal tissues

are better protected from cellular lysis (Bloomfield & Alexander 1967; Kuo & Alexander 1967; Butler & Day 1998a) and are better able to endure extreme variations in moisture and temperature than non-melanised species (Mexal & Reid 1973; Zhdanova et al. 1973; Sealy et al. 1980; Zhdanova et al. 1980; Rehnstrom & Free 1997; Rosas & Casadevall 1997; Pigott 2006; Koide et al. 2014). The ability of melanin to absorb large amounts of water (Sealy et al. 1980) is probably related to these benefits. Melanin appears to slow decomposition in fungal tissues in a way analogous to the way lignin inhibits plant decomposition (Fernandez et al. 2016).

Viable *C. geophilum* may also exhibit greater longevity as it competes well with rhizosphere saprotrophs for soil moisture (Koide & Wu 2003) and nutrients (Orwin et al. 2011), putting these saprotrophs at a disadvantage. This competition reduces the ability of soil saprotrophs to decompose *C. geophilum* (Read & Perez-Moreno 2003; Leake et al. 2004; Read et al. 2004) as they must allocate resources to finding water and nutrients. Given the extent of its exploratory extrametrical hyphae, the dampening effect of *C. geophilum* could be substantial, especially in areas dominated by this species.

Another potential source for the recalcitrance of *C. geophilum* is the presence of toxic metals in its tissues. In addition to indolic and phenolic monomers, melanins may contain peptides, carbohydrates, and hydrocarbons which possess metal binding sites (Gadd 1992). The metals Al, Ni, Zn, Fe, Cu, Cd, and Pb have been found in high concentrations in melanised fungal tissues (Rizzo et al. 1992; Fogarty & Tobin 1996). These toxic concentrations may cause hydrolytic enzymes produced by soil saprotrophs

to be less effective. Metals in such concentrations could also be toxic to predatory soil invertebrates (Rizzo et al. 1992).

In summary, *C. geophilum* mantles did not decompose significantly during the 16 months of observation. As *C. geophilum* is an ultra-generalist (Trappe 1962; Kranabeter 1998; Tedersoo et al. 2003; Dickie & Reich 2005) and is inhabits various horizons of soil (Dickie et al. 2002), its necromass may be considerable, especially in forests with depressed rates of litter decomposition (Fernandez et al. 2015). As melanised fungal cell walls are mineralized more slowly than hyaline fungi (Hurst & Wagner 1969; Malik & Haider 1982), *C. geophilum* may be a large contributor to SOM in forest floors, and may play a large role in carbon sequestration (Fernandez et al. 2013; Fernandez et al. 2016).

Non-melanised mantles with recalcitrant secondary metabolites – Russulaceae & Piloderma

The Russulaceae is a globally distributed family within the basidiomycota and its members are well known for their mycorrhizal associations with various tree species. Species of *Lactarius* and *Russula,* two genera in the Russulaceae, were the most abundant ectomycorrhizal fungi observed after *C. geophilum*. *Piloderma* is also a basidiomycete and is abundant in boreal forest soils (Arocena et al. 2001; Rosling et al. 2003; Lindahl et al. 2007). The ectomycorrhizae and emanating hyphae of *Piloderma fallax* has a characteristic golden-yellow colour caused by the pigment corticrocin (Shreiner et al. 1998) making this species easily identifiable.

Members of *Lactarius, Russula*, and *Piloderma* are not known to possess high concentrations of melanin; however, many species in these genera are known to produce secondary metabolites, some of which could have inhibitory effects on decomposition. A secondary metabolite is any product produced by an organism that is not required in its primary metabolic pathways. These compounds usually have a low molecular weight and are often bioactive in some way (Keller et al. 2005).Various members of the Russulaceae have been shown to produce sesquiterpenes (Ayer & Browne 1981; Daniewski et al. 1993; Daniewski et al. 1995; Clericuzio et al. 1999; Lin & Ji-Kai 2002; Liu 2007), a class of terpenes that consist of several isoprene units (Keller et al. 2005). Sesquiterpenes are highly bioactive (Kramer & Abraham 2012) and thought to play a role in the chemical defence systems of some fungi (Sterner et al. 1985; Daniewski et al. 1993; Luo et al. 2005). Although the sesquiterpenes presented in the above studies were isolated from above-ground sporocarp tissues, these metabolites should also be present in the mycorrhizae formed by these species. In a litter-bag experiment by Koide & Malcolm (2009), *Lactarius chrysorhheus* decomposed least among the tested species, even when compared to the recalcitrant *C. geophilum*. This species of *Lactarius* is known to contain the sesquiterpene velutinal (De Bernardi et al. 1993) which gives it a notably acrid taste. Sesquiterpenes have also been isolated from *Russula* species (Andin et al. 1980; Vidari et al. 1998; Yoshikawa et al. 2006) and may exhibit properties similar to those found in *Lactarius.*

Regarding *Piloderma,* Arocena et al. (2001) describe species belonging to this genus as possessing encrustations of calcium oxalate crystals along their hyphae. The calcium oxalate may act as a hydrophobic coating, which could reduce the risk for microbial attack by dehydrating the hyphae (Whitney & Arnott 1987). The high calcium concentrations needed to form crystals may also inhibit various bacterial enzymes, resulting in a decrease in the decomposition rate of these hyphae (Whitney & Arnott 1988). The crystals may also act as a physical deterrent against predatory invertebrates such as collembola (Thompson 1984; Böllmann et al. 2010). In addition to antifeedant properties, the sequestration of calcium ions from the environment can lead to decreased effectiveness of calcium dependent enzymes produced by some microbes (Whitney & Arnott 1987).

Hyaline ECM – Cortinarius, Hebeloma, and Amanita

Unlike other categories, ECM with hyaline mantles had a gossamer appearance with no apparent pigmentation (Figure 12b). This category showed a rapid decrease over time and was no longer observed after the time interval four. As this category is generally defined by a *lack* of characteristics, it is hard to make conclusions about why these mantles degraded quickly other than that they did not contain melanin or other apparent pigmentation and that most species in these genera are not reported to produce significant amounts of antimicrobial secondary metabolites.

Mantles in this category proved the most difficult to identify due to their lack of recognizable features. The genera listed in this category were suggested based on a combination of microscopic observation and genetic sequencing data on the primary fine

roots used in this study (see Chapter 3). Many mantles in this category exhibited fine extramatrical hyphae. These extensions increase fungal surface area exposed to the soil environment, potentially increasing access to saprotrophic microbes resulting in elevated decomposition rates. Forests dominated by such genera could possibly lose carbon more quickly than forests dominated by recalcitrant fungi such as *C. geophilum,* although this is purely speculative. More research into the decomposition of hyaline ectomycorrhizae is necessary as some of the genera assigned to this category do contain species that produce antifeedant secondary metabolites (*e.g. Amanita* and *Hebeloma*). The rapid disappearance of these mantles may have been due to their small representation in the data set; hyaline mantles made up the smallest group at 3.7% (n = 133) of observed mantles.

Conclusion

As made evident by the varying decomposition rates of ectomycorrhizal fungi, the dynamics of ectomycorrhizal decomposition warrant further investigation as rates of soil organic matter loss of forests to the atmosphere may depend appreciably on dominant ectomycorrhizal partners in that system. It is apparent that fungi with highly melanised mantles such as *C. geophilum* are fairly recalcitrant. Much less is known about the rates of decomposition of non-melanised and hyaline mantles. As the mean average soil temperature of northern forests continues to rise as a result of climate change, increased microbial activity may hasten the decomposition of fungal and fine root tissues. A better understanding of the dynamics of ectomycorrhizal decomposition is necessary to hone the accuracy of carbon models for northern forests.

Future Research

The classification scheme for mantle decomposition used in this study was vulnerable to subjectivity. A biochemical method for describing decomposition level is desirable over visual approximation. This is a challenge as the ectomycorrhizal component of the fine root needs to be quantified separately from the root tissue and from saprotrophic fungi. One solution is to use flourescein diacetate, a viability probe used to identify living cells. Fernandez et al. (2013) used this method in a study on the decomposition of *C. geophilum* mantles. Future experiments could focus on the burial of ectomycorrhizae of different genera in litter bags in different soil environments (Koide et al. 2013). In conjunction with the burial of ectomycorrhizae, bags of green and rooibos tea should also be buried to compare baseline decomposition rates between sites. Large numbers of tea bags can be used per site as they are inexpensive and increase the ability to detect statistically significant differences in decomposition rates between sites with different edaphic properties. Future studies looking would benefit from increased numbers of sites instead of having fewer sites with several plots. As the edaphic properties that affect root litter decomposition vary within a site, having several plots within a site may not elucidate how the site as a whole acts. Rather, sites should be placed in areas with known traits. For example, sample sites should be chosen to represent a range of pH, soil moisture, tree community, and slope position conditions. Due to the edaphic and topographic heterogeneity typical of the Acadian Forest, several plots within one site separated by hundreds of meters may show considerable variation.

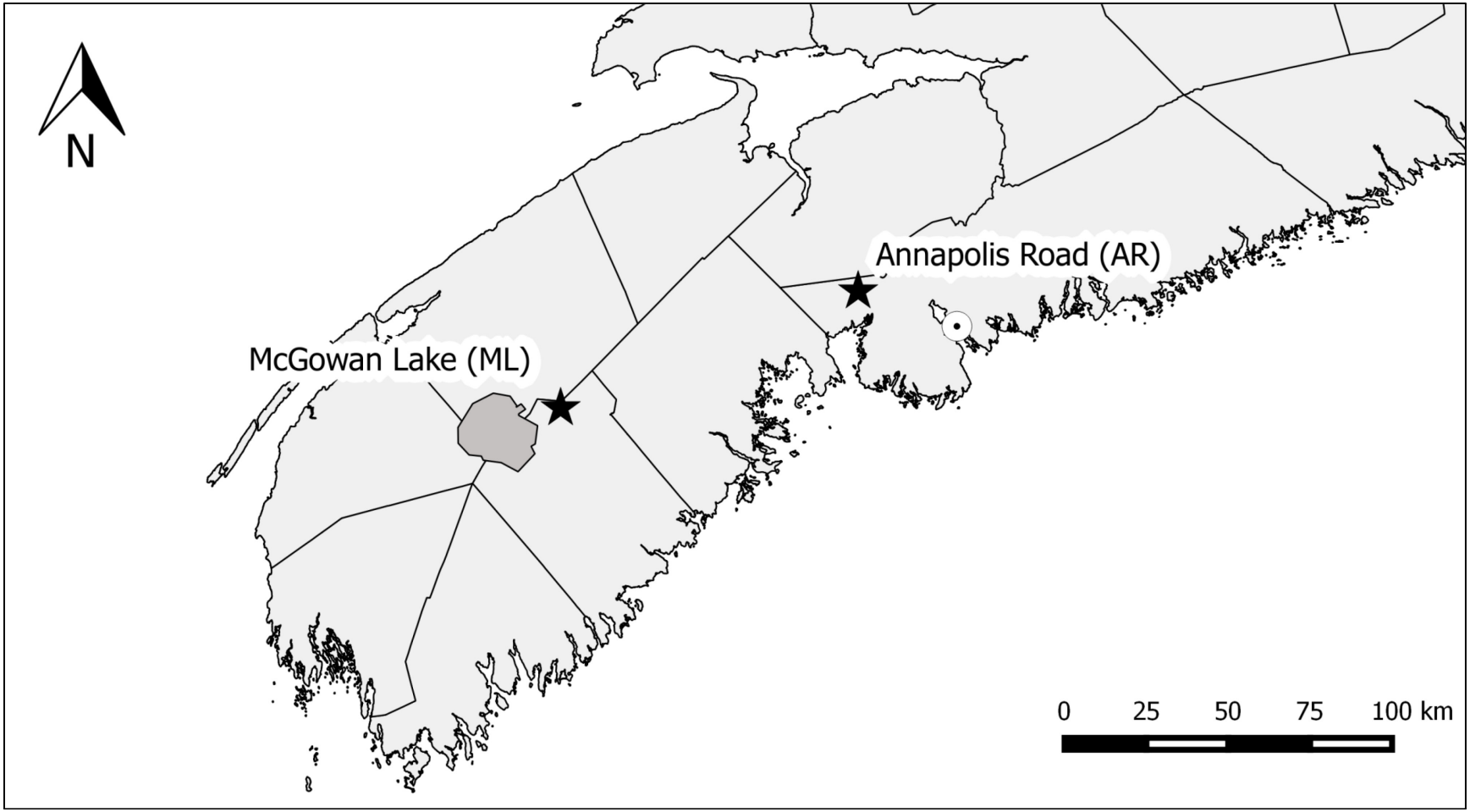

Figure 1. Location of study sites on mainland Nova Scotia: stars = site location; dark grey = Kejimkujik National Park; white circle = Halifax.

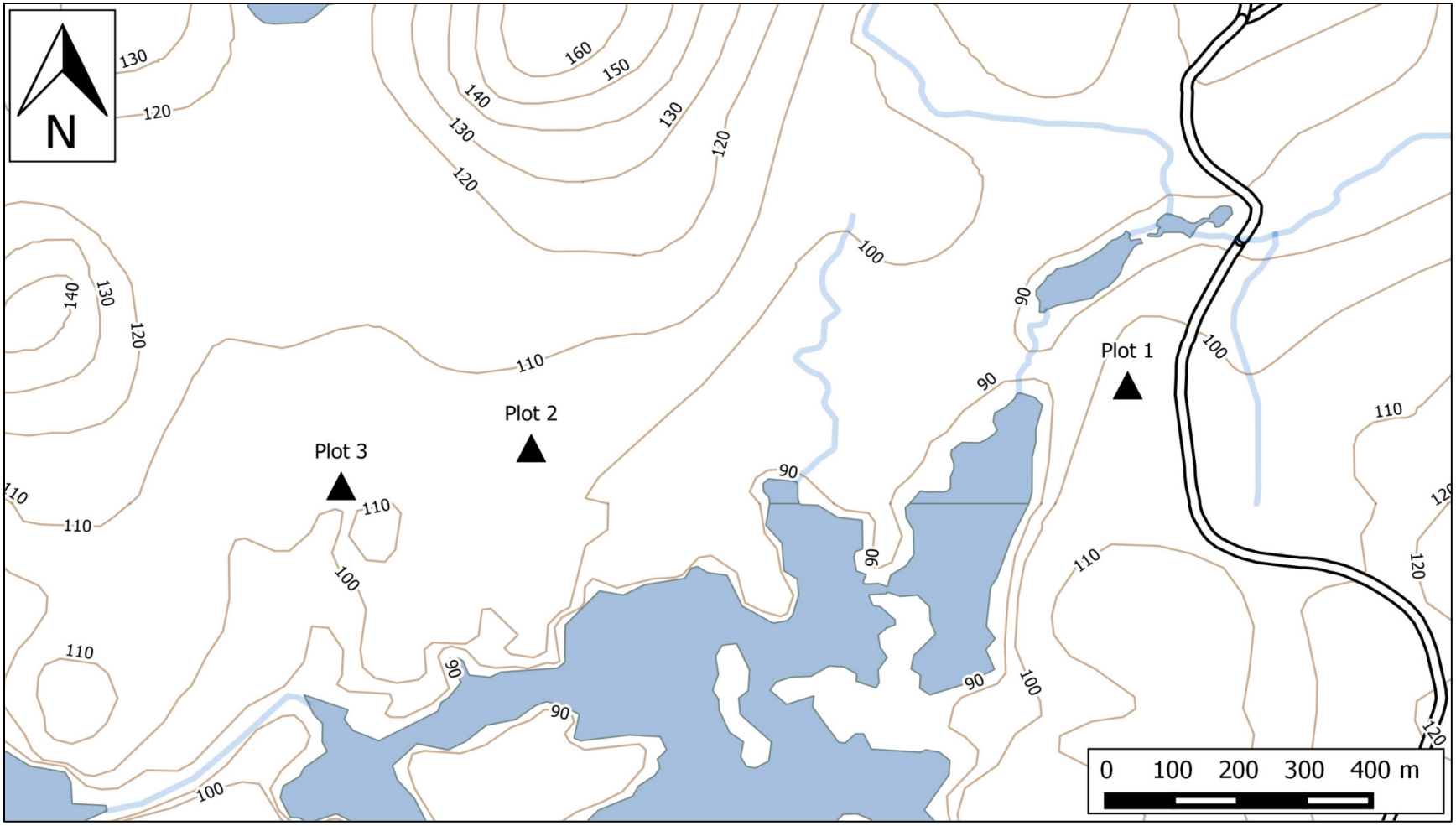

Figure 2. Topography of site at Annapolis Road: filled triangles = plots; contours in meters.

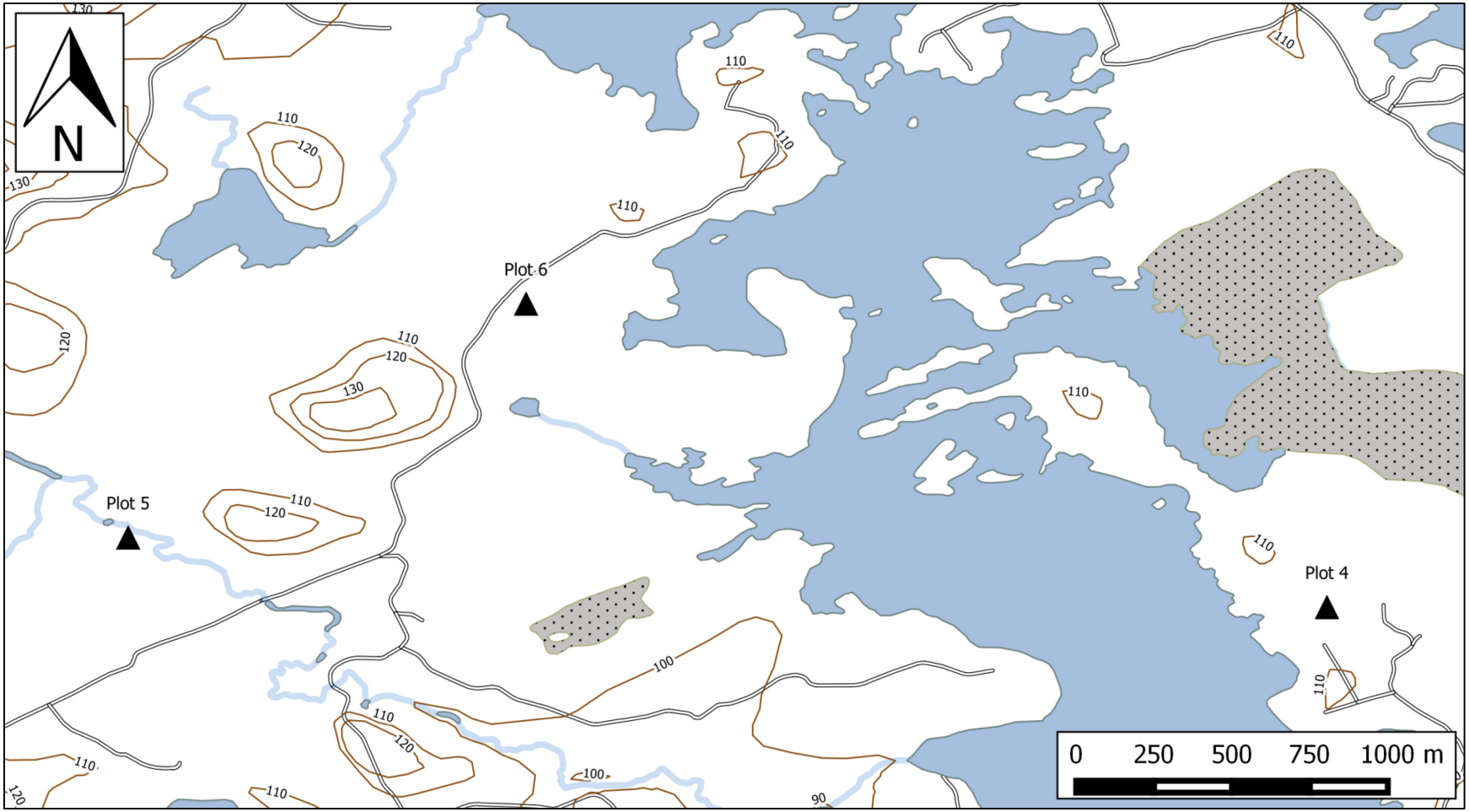

Figure 3. Topography of site at McGowan Lake: filled triangles = plots; dotted grey = bog; contours in meters.

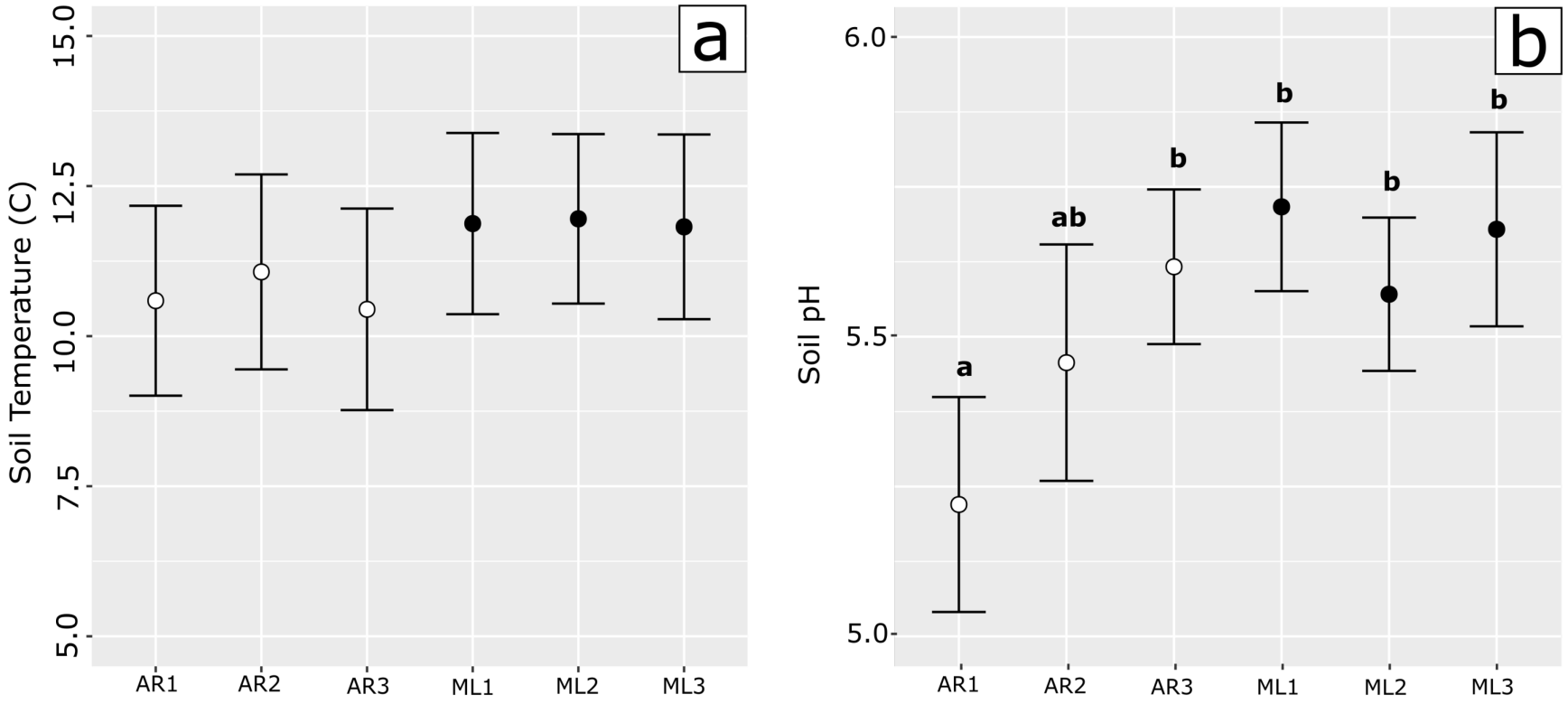

Figure 4. Edaphic measurements by plot: a) soil temperature; b) soil pH; error-bars = 95% confidence intervals.

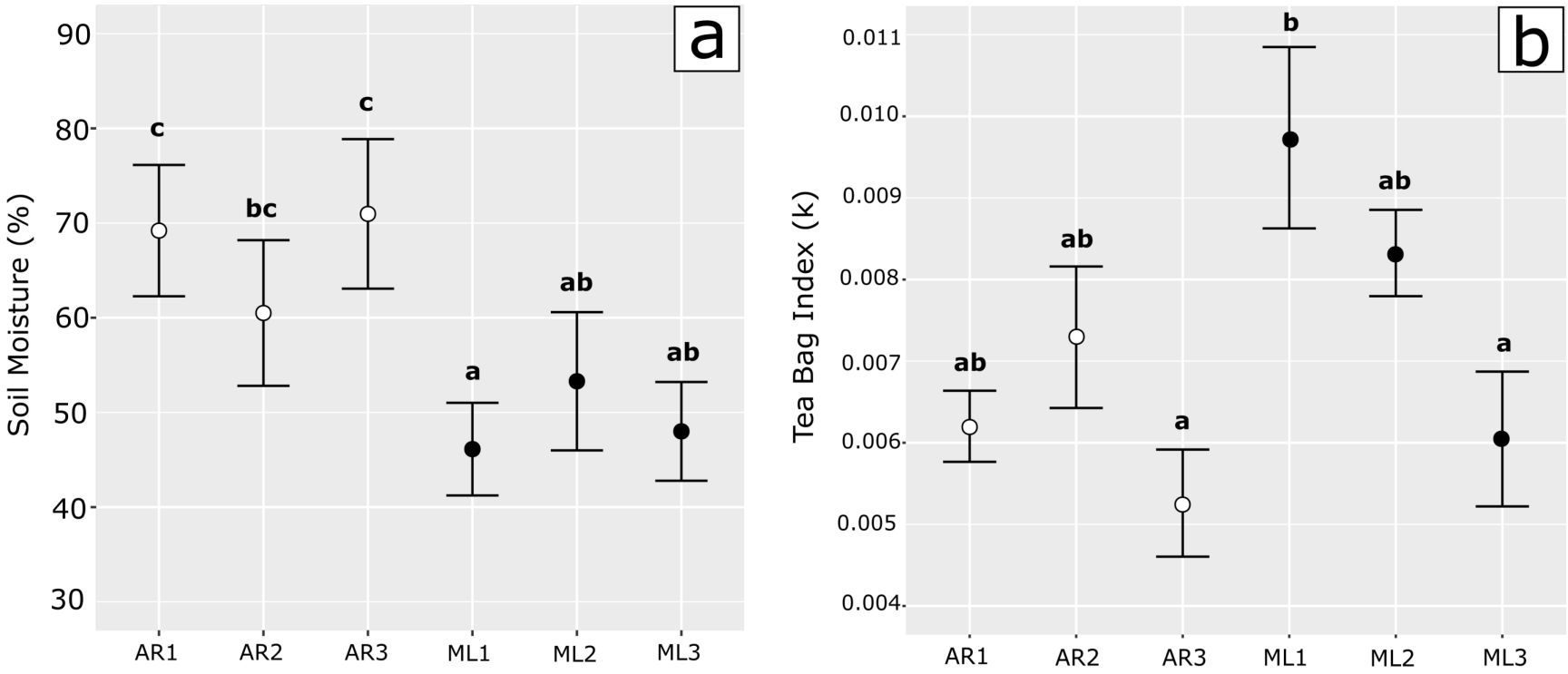

Figure 5. Edaphic measurements by plot: a) soil moisture; b) tea bag index; error-bars = 95% confidence intervals.

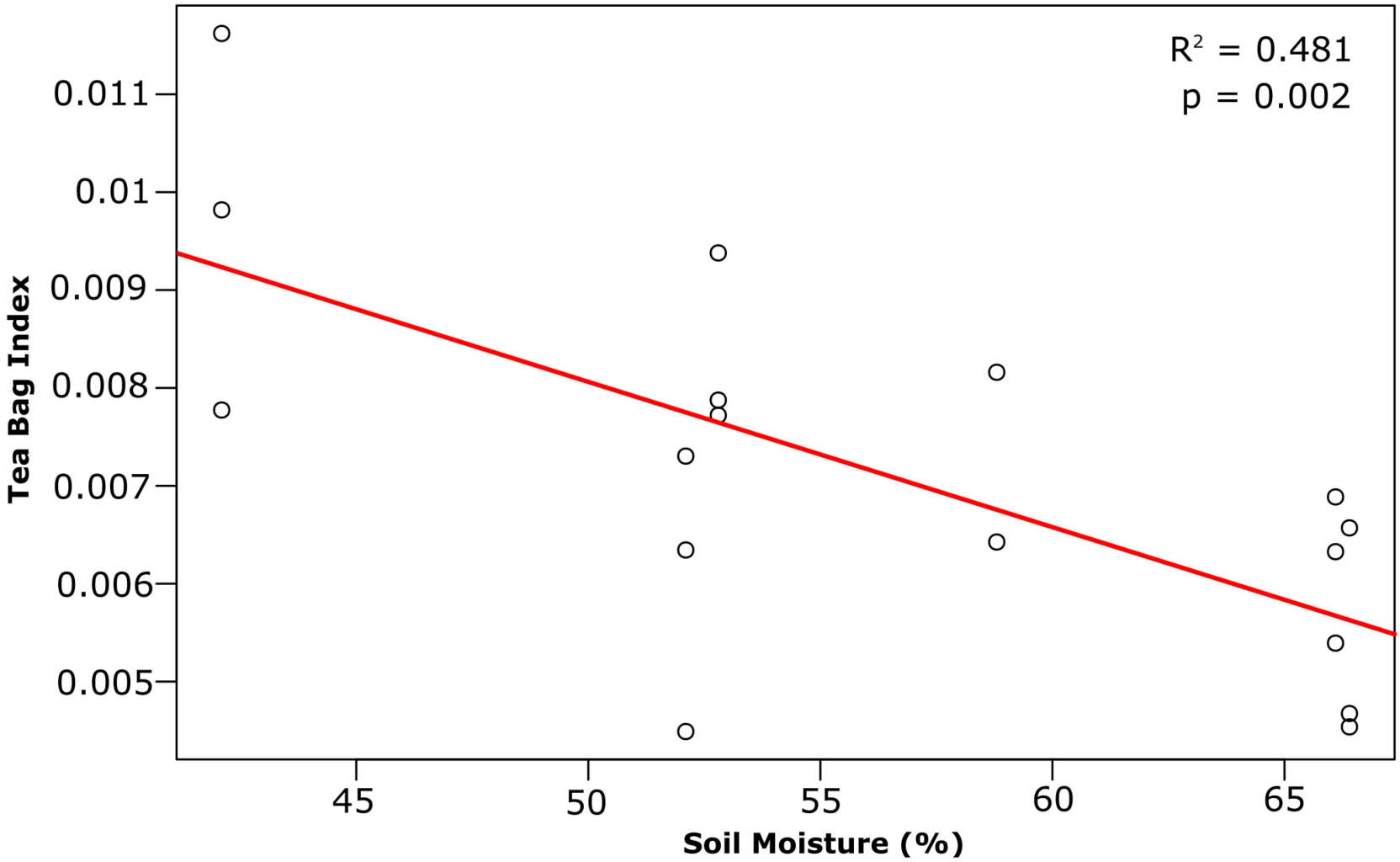

Figure 6. Linear regression of tea bag index against soil moisture.

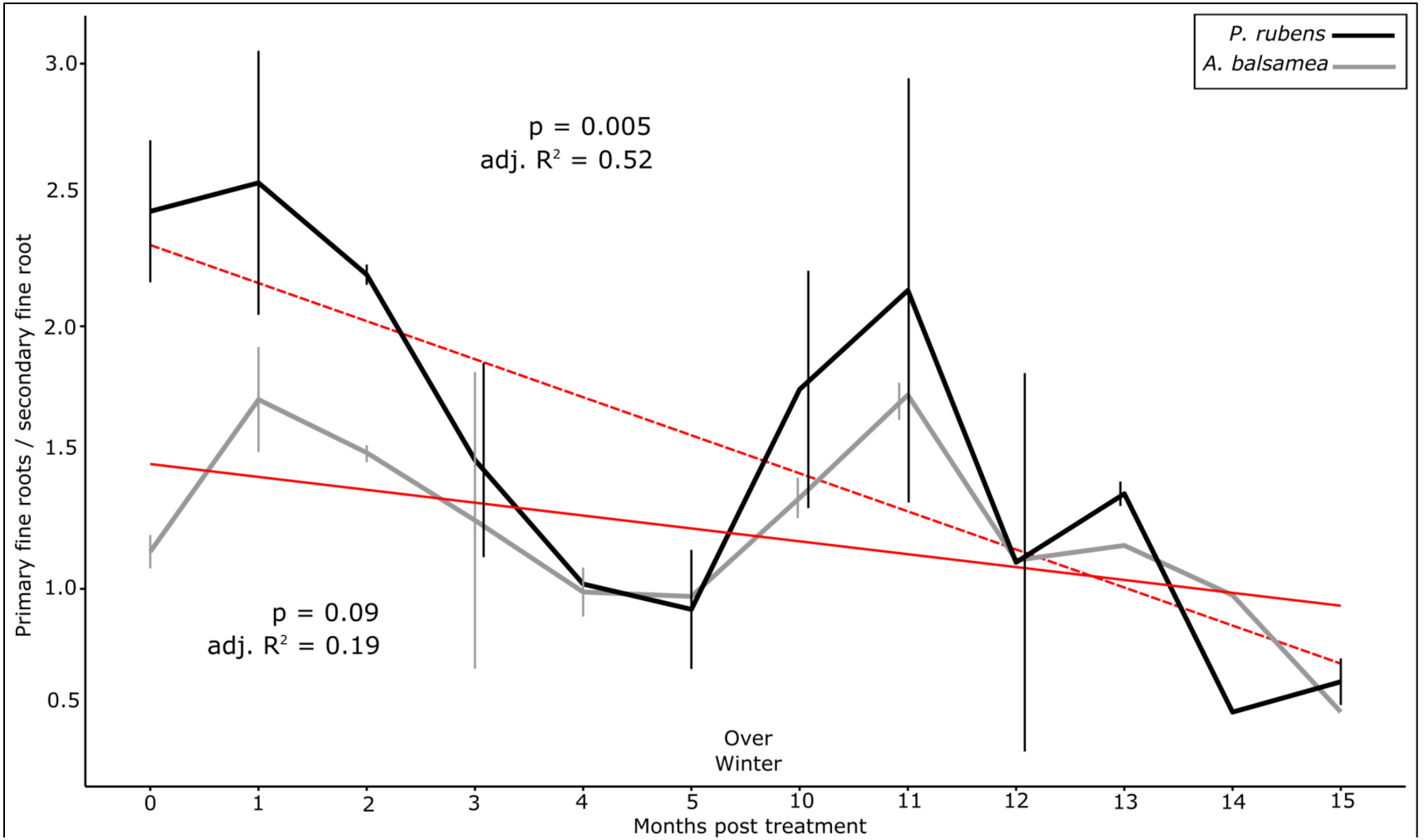

Figure 7. Primary fine root loss in treatment seedlings of *A. balsamea* and *P. rubens*; error-bars = SE.

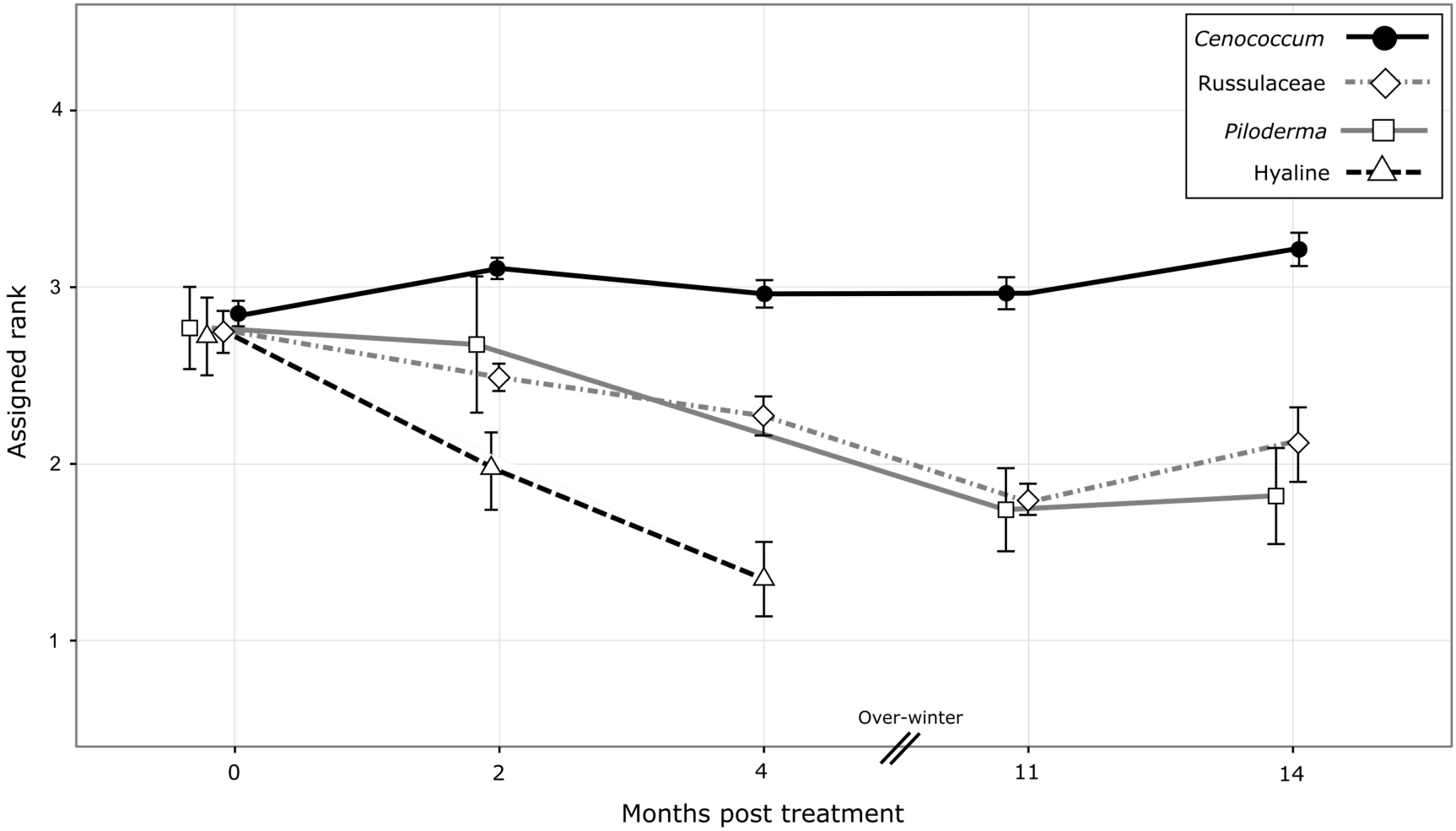

Figure 8. Mean mantle ranking for mantle categories over 14 months; error bars represent 95% confidence intervals.

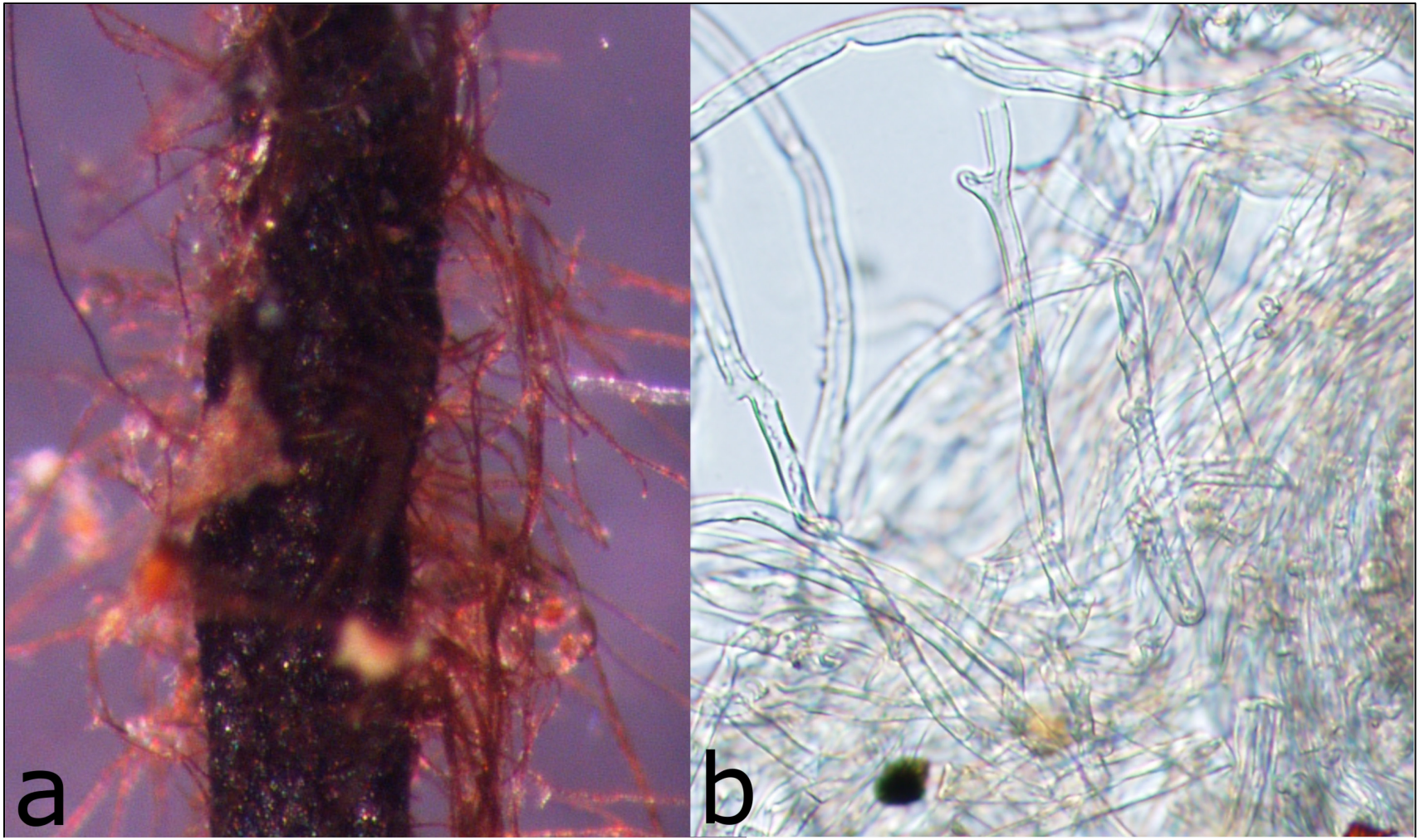

Figure 9. (a) Melanised ectomycorrhiza of C. geophilum; (b) non-melanised hyphae of an unidentified hyaline ectomycorrhiza.

Table 1. Summary of edaphic measurements for plots (± SE) for plots at Annapolis Road (AR) and McGowan Lake (ML).

Site	Plot	Soil pH	Soil Temp. (°C)	Soil Moisture (%)	Tea Bag Index (*k*)
AR	1	5.2 (±0.1)	10.6 (±1.4)	69.2 (±3.4)	7.7e-3 (±3e-4)
	2	5.5 (±0.1)	11.1 (±1.4)	60.5 (±3.8)	8.0e-3 (±1e-3)
	3	5.6 (±0.1)	10.4 (±1.5)	71 (±3.9)	6.3e-3 (±3e-4)
ML	1	5.7 (±0.1)	11.1 (±1.2)	46.1 (±2.4)	9.0e-3 (±1e-3)
	2	5.6 (±0.1)	11.1 (±1.2)	53.3 (±3.5)	8.3e-3 (±9e-4)
	3	5.7 (±0.1)	11.1 (±1.2)	48 (±2.5)	6.7e-3 (±3e-4)

Table 2. Mantle observations from *A. balsamea* and *P. rubens* seedlings at Annapolis Road (AR) and McGowan Lake (ML).

Category	Site	*A. balsamea*	*P. rubens*	Total	%
Cenococcum	AR	907	327	1234	72.4
	ML	590	763	1353	
Russulaceae	AR	316	180	496	19.6
	ML	84	119	203	
Piloderma	AR	36	6	42	4.3
	ML	41	72	113	
Hyaline ECM	AR	54	19	73	3.7
	ML	28	32	60	
Totals		2056	1518	3574	100

Table 3. Proportional odds calculated using ordinal-logit model.

Coefficient	Estimate (β)	SE	p-value
ECM type			
Hyaline	- 1.67	0.17	< 0.001
Piloderma	- 1.07	0.16	< 0.001
Russulaceae	- 1.29	0.08	< 0.001
Site			
McGowan	- 0.21	0.06	< 0.001

Table 4. Pair-wise comparison of seedling age of *A. balsamea* and *P. rubens* across plots.

	Plot					
	-	AR1	AR2	AR3	ML1	ML2
Plot	AR2	0.241	-	-	-	-
	AR3	**< 0.001**	**< 0.001**	-	-	-
	ML1	**0.002**	0.332	0.114	-	-
	ML2	**< 0.001**	0.114	0.332	1	-
	ML3	0.337	1	**< 0.001**	0.152	**0.034**

References

Aber, J. D., Melillo, J. M., Nadelhoffer, K. J., McClaugherty, C. A., & Pastor, J. (1985). Fine root turnover in forest ecosystem in relation to quantity and form of nitrogen availability: a comparison of two methods. *Oecologia* **66**:317-321.

Aerts, R. (1997). Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* **79**:439–449.

Agerer, R. (1987). *Colour atlas of ectomycorrhizae: with glossary*. Einhorn-Verlag: Schwäbisch-Gmünd.

Allen EB, Allen MF, Helm DJ, Trappe JM, Molina RM, Rincon M. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. *Plant and Soil* **170**: 47–62.

Andina, D., de Bernardi, M., del Vecchio, A., Fronza, G., Mellerio, G., Vidari, G., & Vita-Finzi, P. (1980). Sesquiterpenes from Russula sardonia. *Phytochemistry* **19**:93-97.

Aulen, M., Shipley, B., & Bradley, R. (2012). Prediction of in situ root decomposition rates in an interspecific context from chemical and morphological traits. *Annals of Botany* **109**:287-297.

Ayer, W. A., & Browne, L. M. (1981). Terpenoid metabolites of mushrooms and related basidiomycetes. *Tetrahedron* **37**:2197-2248.

Bååth, E., Nilsson, L. O., Göransson, H., & Wallander, H. (2004). Can the extent of degradation of soil fungal mycelium during soil incubation be used to estimate ectomycorrhizal biomass in soil? *Soil Biology and Biochemistry* **36**:2105-2109.

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.

Batjes, N. H. (1996). Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* **47**:151-163.

Berg, B. (1984). Decomposition of root litter and some factors regulating the process: long-term root litter decomposition in a Scots pine forest. *Soil Biology Biochemistry* **16**:609-617.

Berg, B. (2000). Litter decomposition and organic matter turnover in northern forest soils. *Forest Ecology and Management* **133**:13–22.

Birouste, M., Kazakou, E., Blanchard, A., & Roumet, C. (2012). Plant traits and decomposition: are the relationships for roots comparable to those for leaves? *Annals of Botany* **109**:463-472.

Bloomfield, B. J. & Alexander, M. (1967). Melanins and resistance of fungi to lysis. *J. Bacteriol.* **93**:1276-1280.

Böllmann, J., Elmer, M., Wöllecke, J., Raidl, S., & Hüttl, R. F. (2010). Defensive strategies of soil fungi to prevent grazing by Folsomia candida (Collembola). *Pedobiologia* **53**:107-114.

Bowman, S. M., & Free, S. J. (2006). The structure and synthesis of the fungal cell wall. *BioEssays* **28**:799-808.

Bowman, S. M., Piwowar A., Al-Dabbous, M., Vierula, J., & Free, S. J. (2006). Mutational analysis of the GPI anchor pathway demonstrates that GPI anchored

proteins are required for cell wall biogenesis and normal hyphal growth in Neurospora crassa. *Eukaryotic Cell* **5**:187–200.

Butler, M. J., & Day, A. W. (1998a). Fungal melanins: a review. *Can. J. Microbiol.* **44**:1115-1136.

Butler, M. J., & Day, A. W. (1998b). Destruction of fungal melanins by ligninases of Phanerochaete chrysosporium and other white rot fungi. *International journal of plant sciences* **159**:989-995.

Chen H., Harmon, M. E., Sexton, J., & Fasth, B. (2002). Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, U.S.A. *Can. J. For. Res.* **32**:320-331.

Christensen, R. H. B. (2015). Ordinal - Regression Models for Ordinal Data. R package version 2015.6-28.

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

Clericuzio, M., Toma, L., & Vidari, G. (1999). Isolation of a new caryophyllane ester from Lactarius subumbonatus: conformational analysis and absolute configuration. *European journal of organic chemistry* **9**:2059-2065.

Clinton, P. W., Buchanan, P. K., & Allen, R. B. (1999). Nutrient composition of epigeous fungal sporocarps growing on different substrates in a New Zealand mountain beech forest. *New Zealand Journal of Botany* **37**:149-153.

Cooke, R. C., Rayner, A. D. M., & Whipps, J. M. (1993). *Ecophysiology of fungi.* Blackwell Science Publications: Oxford

Cotrufo, M. F., Ineson, P., & Rowland, A. P., (1994). Decomposition of tree leaf litters grown under elevated CO2: effect of litter quality. *Plant and Soil* **163**:121–130.

Cusack, D. F., Chou, W. W., Yang, W. H., Harmon, M. E., & Silver, W. L. (2009). Controls on long-term and leaf litter decomposition in neotropical forests. *Global Change Biology* **15**:1339-1355.

Dahlberg A. 2001. Community ecology of ectomycorrhizal fungi: an advancing interdiciplinary field. *New Phytol.* **150**:555–562.

Dahlman, R. C., & Kucera, C. L. (1965). Root productivity and turnover in native prairie. *Ecology* **46**:84-89.

Daniewski, W. M., Gumulka, M., Ptaszynska, K., Skibicki, P., Bloszyk, E., Drozdz, B., Stromberg, S., Norin, T., & Holub, M. (1993). Antifeedant activity of some sesquiterpenoids of the genus Lactarius (Agaricales: Russulaceae). *European Journal of Entomology* **90**:65-70.

Daniewski, W. M., Gumulka, M., Przesmycka, D., Ptaszynska, K., Bloszyk, E., & Drozdz, B. (1995). Sesquiterpenes of Lactarius origin, antifeedant structure-activity relationships. *Phytochemistry* **38**:1161-1168.

Davidson, E. A. & Jannsens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature Reviews* **440**:165-173.

De Bernardi, M., Garlaschelli, L., Toma, L., Vidari, G., & Vita-Finzi, P. (1993). The chemical basis of hot-tasting and yellowing of the mushrooms Lactarius chrysorrheus and L. scrobiculatus. *Tetrahedron* **49**:1489-1504.

Dickie, I. A., Xu, B., & Koide, R. T. (2002). Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytol.* **156**:527-535.

Dickie, A., & Reich, P. B. (2005). Ectomycorrhizal fungal communities at forest edges. *Journal of Ecology* **93**:244-255.

Dixon, R. K., Brown, S., Houghton, R. A., Solomon, A. M., Trexler, M. C., & Wisniewski, J. (1994). Carbon pools and flux of global forest ecosystems. *Science* **263**:185-191.

Fan, P. P., & Guo, D. L. (2010). Slow decomposition of lower order roots; a key mechanism of root carbon and nutrient retention in the soil. *Oecologia* **163**:509-515.

Fenn, M. (1991). Increased site fertility and litter decomposition rate in high-pollution sites in the San Bernardino Mountains. *Forest Science* **37**:1163–1181.

Fernandez, C. W., Langley, J. A., Chapman, S., McCormack, M. L., & Koide, R. T. (2016). The decomposition of ectomycorrhizal fungal necromass. *Soil Biology and Biochemistry* **93**:38-49.

Finér, L., & Laine, J. (1998). Root dynamics at drained peatland sites of different fertility in southern Finland. *Plant and Soil* **201**:27-36.

Finér, L., Ohasi, M., Noguchi, K., & Hirano, Y. (2011). Fine root production and turnover in forest ecosystems in relation to stand and environmental characteristics. *Forest Ecology and Management* **262**:2008-2023.

Fogarty, R. V., & Tobin, J. M. (1996). Fungal melanins and their interactions with metals. *Enzyme and Microbial Technology* **19**:311-317.

Fujii, S., & Takedai, H. (2010). Dominant effects of litter substrate quality on the difference between leaf and root decomposition process above- and belowground. *Soil Biology & Biochemistry* **42**:2224-2230.

Fleet, G. H. (1991). *The yeast*. 2nd ed. Academic Press: London.

Gadd, G. M. (1992). Interactions of fungi with toxic metals. *New Phytol.* **124**:25-60.

Gill, R. A. & Jackson, R. B. (2000). Global patterns of root turnover for terrestrial ecosystems. *New Phytol.* **147**:13-31.

Goebel, M., Hobbie, S. E., Bulaj, B., Zadworny, M., Archibald, D. D., Oleksyn, J., Reich, P. B., & Eissenstat, D. M. (2011). Decomposition of the finest root branching orders: linking belowground dynamics to fine-root function and structure. *Ecological Monographs* **81**:89-102.

Hendricks, J. J., Hendrick, R. L., Wilson, C. A., Mitchell, R. J., Pecot, S. D., & Guo, D. (2006). Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *Ecology* **94**:40-57.

Hobbie, E. A. (2006). Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology* **87**:563-569.

Hobbie, S. E., Oleksyn, J., Eissenstat, D. M., & Reich, P. B. (2010). Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. *Oecologia* **162**:505-513.

Hurst, H. M., & Wagner, G. H. (1969). Decomposition of 14C-labeled cell wall and cytoplasmic fractions from hyaline and melanic fungi. *Soil Science Society of America Journal* **33**:707-711.

Jackson, J. B., Mooney, H. A., & Schulze, E. D. (1997). A global budget for fine root biomass, surface area, and nutrient contents. *Proc. Natl. Acad. Sci.* **94**:7362-7366.

Joslin, J. D., Wolfe, M. H., & Hanson, P. J. (2000). Effects of altered water regimes on forest root systems. *New Phytol.* **147**:117-129.

Joslin, J. D., Gaudinski, J. B., Torn, M. S., Riley, W. J., & Hanson, P. J. (2006). Fine root turnover patterns and their relationships to root diameter and soil depth in a ^{14}C-labeled Deciduous forest. *New Phytol.* **172**:523-535.

Kapteyn, J. C., Van Den Ende, H., & Klis, F. M. (1999). The contribution of cell wall proteins to the organization of the yeast cell wall. *Biochimica et Biophysica Acta* **1426**:373-383.

Kätterer, T., Bolinder, M. A., Andrén, O., Kirchmann, H., & Menichetti, L. (2011). Roots contribute more to refractory soil organic matter than aboveground crop residues as revealed by a long-term field experiment. *Agriculture Ecosystems and Environment* **141**:184-192.

Keller, N. P., Turner, G., & Bennett, J. W. (2005). Fungal secondary metabolism—from biochemistry to genomics. *Nature Reviews Microbiology* **3**:937-947.

Keuskamp, J. A., Dingemans, B. J. J., Lehtinen, T., Sarneel, J. M., & Hefting, M. M. (2013). Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. *Methods in Ecology and Evolution* **4**:1070-1075.

Keyes, M. R., & Grier, C. C. (1981). Above- and below-ground net production in 40-year-old Douglas-fir stands on low and high productivity sits. *Can. J. For. Res.* **11**:599-605.

Koide, R. T., & Malcolm, G. M. (2009). N concentration controls decomposition rates of different strains of ectomycorrhizal fungi. *Fungal Ecology* **2**:197-202.

Koide, R. T., Fernandez, C. W., & Peoples, M. S. (2011). Can ectomycorrhizal colonization of Pinus resinosa roots affect their decomposition? *New Phytol.* **191**:508-514.

Koide, R. T., & Malcolm, G. M. (2009). N concentration controls decomposition rates of different strains of ectomycorrhizal fungi. *Fungal Ecology* **2**:197-202.

Koide, R. T., Fernandez, C., & Malcolm, G. (2014). Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytol.* **201**:433-439.

Kramer, R., & Abraham, W-R. (2012). Volatile sesquiterpenes from fungi: what are they good for? *Phytochem. Rev.***11**:15-37.

Klis, F. M. (1994). Review: cell wall assembly in yeast. *Yeast* **10**:851–869.

Klis, F. M., Mol, P., Hellingwerf, K., & Brul, S. (2002). Dynamics of cell wall structure in Saccharomyces cerevisiae. *FEMS Microbiol. Rev*. **26**:239–256.

Kranabetter, J. M., & Wylie, T. (1998). Ectomycorrhizal community structure across forest openings on naturally regenerated western hemlock seedlings. *Canadian Journal of Botany* **76**:189-196.

Krywolap, G. N. (1964). Cenococcum graniforme antibiotics: elaboration in pure culture and during mycorrhizal association (Doctoral dissertation).

Kuo, M. J., & Alexander, M. (1967). Inhibition of the lysis of fungi by melanins. *Journal of Bacteriology* **94**:624-629.

Lahti, M., Aphalo, P. J., Finér, L., Ryyppö, Lehto, T., & Mannerkoski, H. (2005). Effects of soil temperature on shoot and root growth and nutrient uptake of 5-year-old Norway spruce seedlings. *Tree Physiology* **25**:115-122.

Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science* **304**:1623-1627.

Langley, J. A., & Hungate, B. A. (2003). Mycorrhizal controls on belowground litter quality. *Ecology* **84**:2302-2312.

Langley, J. A., Chapman, S. K., & Hungate, B. A. (2006). Ectomycorrhizal colonization slows root decomposition: the post-mortem fungal legacy. *Ecology Letters* **9**:955-959.

Leake, J., Johnson, D., Donnelly, D., Muckle, G., Boddy, L., & Read, D. (2004). Networks of power and influence: the role of mycorrhizal mycelium in

controlling plant communities and agroecosystem functioning. *Canadian Journal of Botany* **82**:1016-1045.

Lin, H., & Ji-Kai, L. (2002). The first humulene type sesquiterpene from Lactarius hirtipes. *Zeitschrift für Naturforschung* C **57**:571-574.

Lin, C., Yang, Y., Guo, J., Chen, G., & Xie, J. (2011). Fine root decomposition of evergreen broadleaved and coniferous tree species in mid-subtropical China: dynamics of dry mass, nutrient and organic fractions. *Plant Soil* **338**:311-327.

Liu, J. K. (2007). Secondary metabolites from higher fungi in China and their biological activity. *Drug Discov. Ther.* **1**:94-103.

Lindahl, B. D., Ihrmark, K., Boberg, J., Trumbore, S. E., Högberg, P., Stenlid, J., & Finlay, R. D. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.* **173**:611-620.

Lukac, M. (2012). *Measuring Roots: An Updated Approch*. Springer-Verlag: Berlin-Heidelberg.

Luo, D-Q., Wang, F., Bian, X-Y., & Liu, J-K. (2005). Rufuslactone, a new antifgunal sesquiterpenes from the fruiting bodies of the basidiomycete Lactarius rufus. *J. Antibiot.* **58**:456-459.

Malik, K. A., & Haider, K. (1982). Decomposition of 14 C-labelled melanoid fungal residues in a marginally sodic soil. *Soil Biology and Biochemistry* **14**:457-460.

Mandyam, K., & Jumpponen, A. (2005). Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Studies in Mycology* **53**:173-189.

McClaugherty, C. A., Aber, J. D., & Melillo, J. M. (1982). The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. *Ecology* **63**:1481-1490.

McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., Helmisaari, H-S., Hobbie, E. A., Iversen, C. M., Jackson, R. B., Leppälammi-Kujansuu, J., Norby, R. J., Phillips, R. P., Pregitzer, K. S., Pritchard, S. G., Rewald, B., & Zadworny, M. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* **207**:505-518.

Melillo, J. M., Aber, J. D., & Muratore, J. F. (1982). Nitrogen and lignin control of Deciduous leaf litter decomposition dynamics. *Ecology* **63**:621-626.

Mexal, J., & Reid, C. P. P. (1973). The growth of selected mycorrhizal fungi in response to induced water stress. *Canadian Journal of Botany* **51**:1579-1588.

Ostertag, R., & Hobbie, S. E. (1999). Early stages of root and leaf decomposition in Hawaiian -forests: Effects of nutrient availability. *Oceologia* **121**:564-573.

Paris, F., Dexheimer, J., & Lapeyrie, F. (1993). Cytochemical evidence of a fungal cell wall alteration during infection of Eucalyptus roots by the ectomycorrhizal fungus Cenococcum geophilum. *Archives of microbiology* **159**:526-529.

Pigott, C. D. (1982). Survival of mycorrhiza formed by Cenococcum geophilum Fr. in dry soils. *New Phytol.* **92**:513-517.

Pregitzer, K. S., King, J. S., Burton, A. J., & Brown, S. E. (2000). Responses of tree fine roots to temperature. *New Phytol.* **147**:105-115.

Prescott, C. E. (2010). Litter decomposition: what controls it and how we can alter it to sequester more carbon in forest soils? *Biogeochemistry* **101**:133-149.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Read, D. J., & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance? *New Phytol.* **157**:475-492.

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.

Rehnstrom, A. L., & Free, S. J. (1996). The isolation and characterization of melanin-deficient mutants of Monilinia fructicola. *Physiological and molecular plant pathology* **49**:321-330.

Rizzo, D. M., Blanchette, R. A., & Palmer, M. A. (1992). Biosorption of metal ions by Armillaria rhizomorphs. *Canadian Journal of Botany* **70**:1515-1520.

Rohlfs, M., Albert, M., Keller, N. P., & Kempken, F. (2007). Secondary chemicals protect mould from fungivory. *Biology Letters* **3**:523-525.

Rosas, Á. L., & Casadevall, A. (1997). Melanization affects susceptibility of Cryptococcus neoformans to heat and cold. *FEMS microbiology letters* **153**:265-272.

Rosling, A., Landeweert, R., Lindahl, B. D., Larsson, K. H., Kuyper, T. W., Taylor, A. F. S., & Finlay, R. D. (2003). Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New Phytol.* **159**:775-783.

Rudawska, M., & Leski, T. (2005). Macro-and microelement contents in fruiting bodies of wild mushrooms from the Notecka forest in west-central Poland. *Food chemistry* **92**:499-506.

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature* **478**:49-56.

Schreiner, T., Hildebrandt, U., Bothe, H., & Marner, F. J. (1998). Chemical and biological characterization of corticrocin, a yellow pigment formed by the ectomycorrhizal fungus Piloderma croceum. *Zeitschrift für Naturforschung C* **53**:4-8.

Sealy, R. C., Felix, C. C., Hyde, J. S., & Swartz, H. M. (1980). Structure and reactivity of melanins: influence of free radicals and metal ions. *Free radicals in biology* **4**:209-259.

Silver, W. L. & Miya, R. K. (2001). Global patterns in root decomposition: comparisons of climate and litter quality effects. *Oecologia* **129**:407-419.

Solly, E. F., Schöning, I., Boch, S., Kandeler, E., Marhan, S., Michalzik, B., Müller, J., Zscheischler, J., Trumbor, S. E., & Schrumpf, M. (2014). Factors controlling

decomposition rates of fine root litter in temperate forests and grasslands. *Plant Soil* **382**:203-218.

Sterner, O., Bergman, R., Kihlberg, J., & Wickberg, B. (1985). The sesquiterpenes of Lactarius vellereus and their role in a proposed chemical defense system. *Journal of Natural Products* **48**:279-288.

Sun, T., Mao, Z., & Han, Y. (2013). Slow decomposition of very fine roots and some factors controlling the process: a 4-year experiment in four temperate tree species. *Plant Soil* **372**:445-458.

Swift, J. M., Heal, O. W., & Anderson, J. M. (1979). *Decomposition in terrestrial ecosystems*. University of California Press: Oakland.

Taylor, B.R., Parkinson, D., & Parsons, W. F. J., (1989). Nitrogen and lignin content as predictors of litter decay rates, a microcosm test. *Ecology* **70**:97–104.

Tedersoo, L., Kõljalg, U., Hallenberg, N., & Larsson, K. H. (2003). Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytol*. **159**:153-165.

Thompson, W. (1984). Distribution, development and functioning of mycelial cord systems of decomposer basidiomycetes of the deciduous woodland floor. In Symposium series-British Mycological Society.

Tingey, D. T., Phillips, D. L., Johnson, M. G., Rygiewicz, P. T., Beedlow, P. A., & Hogsett, W.E. (2005). Estimates of Douglas-fir fine root production and mortality from minirhizotrons. *Forest Ecology and Management* **204**:359-370.

Tisdal, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. *Journal of Soil Science* **33**:141-163.

Trappe, J. M. (1962). Cenococcum graniforme - its distribution, ecology, mycorrhiza formation, and inherent variation (Doctoral dissertation).

Trofymow, J. A., Morley, C. R., Coleman, D. C., & Anderson, R. V. (1983). Mineralization of cellulose in the presence of chitin and assemblages of microflora and fauna in soil. *Oecologia* **60**:103–110.

Okafor, N. (1966). Estimation of the decomposition of chitin in soil by the method of carbon dioxide release. *Soil Science* **102**:140-142.

Vidari, G., Che, Z., & Garlaschelli, L. (1998). New nardosinane and aristolane sesquiterpenes from the fruiting bodies of Russula lepida. *Tetrahedron letters* **39**:6073-6076.

Vogt, K. A., Moore, E. E., Vogt, D. J., Redlin, M. J., & Edmonds, R. L. (1983). Conifer fine root and mycorrhizal root biomass within the forest floors of Douglas-fir stands of different ages and site productivities. *Can. J. For. Res.* **13**:429-437.

Vogt, K. A., Grier, C. C., & Vogt, D. J. (1986). *Production, turnover, and nutrient dynamics of above and belowground detritus of world forests*. Academic Press: Cambridge.

Vogt, K. A., Vogt, D. J., Moore, E. E., Fatuga, B. A., Redlin, M. A., & Edmonds, R. L. (1987). Conifer and angiosperm fine-root biomass in relation to stand age and site productivity in Douglas-fir forests. *Journal of Ecology* **75**:857-870.

Vogt, K. A., Vogt, D. J., Palmiotto, P. A., Boon, P., O'Hara, J., & Asbjornsen, H. (1996). Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant and Soil* **187**:159-219.

Whitney, K. D., & Arnott, H. J. (1987). Calcium oxalate crystal morphology and development in Agaricus bisporus. *Mycologia* **2**:180-187.

Whitney, K. D., & Arnott, H. J. (1988). The effect of calcium on mycelia growth and calcium oxalate crystal formation in Gibbertella periscaria (Mucorales). *Mycologia* **80**:707-715.

Xiong, Y., Fan, P., Fu, S., Zeng, H., & Guo, D. (2013). Slow decomposition and limited nitrogen release by lower order roots in eight Chinese temperate and subtropical trees. *Plant Soil* **363**:19-31.

Yoshikawa, K., Kaneko, A., Matsumoto, Y., Hama, H., & Arihara, S. (2006). Russujaponols AF, illudoid sesquiterpenes from the fruiting body of Russula japonica. *Journal of Natural Products* **69**:1267-1270.

Zhdanova, N. N., Melezhik, A. V., & Vasilevskaya, A. I. (1980). Thermostability of some melanin-containing fungi. *Biol. Bull. Acad. Sci. U.S.S.R.* **7**:305–310.

Zhdanova, N. N., & Pokhodenko, V. D. (1973). The possible participation of a melanin pigment in the protection of the fungus cell from desiccation. *Microbiology* **42**:753-757.

CHAPTER 3

Molecular Characterisation of Fine Root Associated Fungal Communities during the Early Stages of Decomposition in the Acadian Forest

Abstract

Fine roots are a nexus for different functional groups of fungi. Due to the varying types of relationships between fine root and fungi, the response of fungal communities to host death may shed light on which groups are responsible for decomposition. Seedlings of *Abies balsamea* and *Picea rubens* were killed and left to decompose over a 16 month period at two sites in Acadian Forest. Primary fine roots from various time intervals had their fungal communities amplified via PCR and sequenced. Most fungal species found in control seedlings were ectomycorrhizal. Approximately 3 to 6 months post treatment, endophytic fungi increased in abundance relative to ectomycorrhizal species. This increase begins to recede with ectomycorrhizal fungi becoming dominant once again. Few saprotrophic species were identified, implying that the decomposition of coniferous primary fine roots does not start appreciably during the first 16 months after fine root senescence in the Acadian forest.

Introduction

The fungi represent an extremely diverse branch of life with myriad forms and functions. Many species are cryptic, only recently discernible through molecular techniques. Because of their evolutionary history, the life cycles of most modern fungi involve interactions with plants. Fungi can be grouped into four categories based on these relationships: 1) mycorrhizal - mutualistic symbionts involved in beneficial relationships with the fine roots of plants; 2) endophytic - often commensal organisms that inhabit intercellular regions of plant tissue; 3) saprotrophic – fungi that decompose senescent plant materials and recycle sequestered nutrients; and 4) pathogenic - fungi that cause observable damage to plant tissues. The first of these categories, the mycorrhizal fungi, present a unique plant-fungus relationship. Although fungi belonging to the other functional categories may appear throughout the plant, ectomycorrhizae are hybrids of

plant and fungal tissues only found on fine roots. Fine roots are sites of high metabolic activity (Pregitzer et al. 1995; Norby & Jackson 2000; Tierney & Fahey 2002; Baddeley & Watson 2005; Majdi et al. 2005; Grünig et al. 2008; Kernaghan 2013) and their immediate surroundings (*i.e.* the rhizosphere) can be inhabited by hyper-diverse fungal communities (Fierer et al. 2007; Buee et al 2009; Jumpponen & Jones 2009).

As with any generalization in biology, there can be notable overlap between these groups preventing confident assignment to one group. Although mycorrhizal fungi are described as an intricate form of mutualism, some mycorrhizal fungi retain genes for enzymes used in lignocellulose degradation, a common trait of saprotrophic and pathogenic fungi. Such shifts from mutualism to saprotrophy highlight the spectrum of plant-fungal relationships.

Mycorrhizal Fungi

The majority of plants form mutualistic relationships with mycorrhizal fungi (Smith & Read 1997; Opik et al. 2006). Mycorrhizal fungi enter mutualistic symbioses with plants through their fine roots by developing a nutrient exchange structure. Photosynthetically fixed carbon compounds are provided by the plant host to the fungal symbionts and are the only source of carbon for the primary metabolism of the fungal partner. In return for carbon, plant hosts receive increased access to soil nutrients and water, boosted defense against pathogens, and protection from heavy metals (Marschner & Dell 1994; Schützendübel & Polle 2002; Peterson & Massicotte 2004; Vogel-Mikuš et al. 2004; Whipps 2004; Hildebrandt et al. 2007). Although there are several types of mycorrhizal fungi, this study examines only ectomycorrhizal fungi.

In ectomycorrhizae, a structure known as the Hartig net develops. It is a network of branching hyphae that enter the intercellular spaces between the plant cells of the fine roots (Smith & Read 1997; Peterson & Massicotte 2004; Brundrett 2007). The surface of a colonised fine root is also covered by hyphal cells from the ectomycorrhizal fungus. This layer of fungal tissue is known as the mantle and varies greatly between ectomycorrhizal fungi. Ectomycorrhizal fungi are predominantly basidiomycetes (Peterson & Massicotte 2004), with the Agaricales being especially well represented. This being said, ascomycetous ectomycorrhizal species are not uncommon (*e.g. C. geophilum*). Ectomycorrhizal symbioses are found mostly on woody plants whereas other types of mycorrhizae are found on herbaceous plants.

Endophytic Fungi

The word endophyte means "within the plant"; therefore, any organism living inside a plant can technically fall under this broad definition (Schulz & Boyle 2005; Schulz & Boyle 2006). Although there is debate on what the term endophyte entails, the general consensus is that fungal endophytes live within plant tissues and are detectable but do not cause visible detrimental effects (Schulz & Boyle 2005; Schulz & Boyle 2006; Mayerhofer et al. 2013a). The first reports of fungal endophytes occurred in the early 20th century (Gallaud 1905; Peyronel 1922; Melin 1923). Endophytes can be found in any plant tissue, including the root system. Root endophytes are typically ascomycetes, with many belonging to the Dermateaceae and Helotiaceae (Kernaghan & Patriquin 2011; Kernaghan & Patriquin 2015).

Root endophytes differ from mycorrhizal fungi in that they lack structures for absorptive nutrient transfer, are not restricted to the fine roots and show lower host specificity (Kernaghan 2013). Although root endophytes have been known since the 20th century, study of these organisms has improved with the advent of modern microscopy and molecular techniques. Much is left to be discovered regarding their life histories, functionality, and organismal biochemistry (Jumpponen & Trappe 1998; Upson et al. 2009). Some researchers hypothesize that endophytes live in an antagonistic balance where endophytic virulence and host defence responses eventually result in an asymptomatic colonisation (Schulz & Boyle 2006). However, the lack of baseline knowledge makes it difficult to generalize about endophytic relationships. This is further complicated as many pathogenic species of fungi have stages that are considered endophytic - a problem arising from the definition of endophyte being based on physical rather than functional criteria. These observations need to be taken into consideration when classifying fungi as endophytes.

Saprotrophic Fungi

Many of the fungi present in the rhizosphere are saprotrophic, meaning that they break down the structural components of deceased organisms, releasing sequestered nutrients (Dighton 2003). Rhizosphere saprotrophs are different from pathogens in that they do not attack living tissue (Kernaghan 2013). These fungi are fundamental members of terrestrial ecosystems as they digest the senescent plant tissues consistently being input into soil systems. Saprotrophs convert lignocellulose into molecules that other organisms may utilise in their own metabolic processes and break down recalcitrant plant

compounds by secreting suites of hydrolytic enzymes, absorbing the digested products via active transport across their membranes. Extracellular digestion of organic matter is less efficient than other forms, and nutrients spill-over into the surrounding soil system where they are utilised by other organisms. This fertilization effect makes decomposition by saprotrophs essential, both by liberating sequestered nutrients and removing necromass (Dighton 2003).

The classification of a fungus as a saprotroph can also be problematic, mycorrhizal and endophytic species may also have the ability to decompose senescent plant tissue. This confusion aside, there are some groups of fungi that are well characterized as being almost entirely saprotrophic, including members of the zygomycete genera *Mortierella* and *Umbelopsis*, and the hyphomycete genera *Cylindrocarpon, Penicillium, Trichoderma,* and *Fusarium* (Kernaghan 2013)

Pathogenic Fungi

Fungal root pathogens are infectious organisms causing observable disease in plant roots, *i.e.* killing root cells to extract compounds to nourish themselves. Fungal root pathogens can be divided into biotrophic and necrotrophic species depending on their life style. Biotrophic pathogens require living cells (and therefore a living host) to obtain nutrients, whereas necrotrophic pathogens derive their energy from dead cells (Lewis 1973; Raajimakers et al. 2009). Biotrophs also exhibit much narrower host ranges than necrotrophs (Lewis 1973; Raajimakers et al. 2009). Although the term biotroph is widely used, many fungi are technically hemibiotrophic, as they begin as biotrophs but become necrotrophic later in their life cycle (Perfect & Green 2001; Oliver & Ipcho 2004).

Under certain conditions, a fungal root pathogen will extend its hyphae toward the cells of a root and attach itself to the epidermis (Raajimakers et al. 2009). Once on the root epidermis, biotrophs produce haustoria (Perfect & Green 2001) whereas necrotrophs kill root epidermal cells in advance of their approaching hyphae via production of phytotoxins and hydrolytic enzymes (Raajimakers 2009; Kernaghan 2013). Once inside the plant, necrotrophs ramify between cells and colonize the root cortex in the process (Raajimakers et al. 2009) whereas biotrophs are show less aggressive behaviour.

Fine Root Decomposition

Trees dedicate 60-70% of their net primary productivity (NPP) to below-ground tissues and metabolic processes (Vogt et al. 1982; Vogt et al. 1995; Fogel & Hunt 1983; Finlay & Söderström 1992; Hendrick & Pregitzer 1992; Nadelhoffer & Raich 1992; Ryan et al. 1996). Approximately half of this NPP is used to replace fine roots even though they only represent ~ 2.5% of terrestrial plant biomass (Jackson et al. 1997). Although some of this is allocated for metabolic functions in healthy fine roots, a large part is used to replace tissues lost to the continuous process of fine root turnover; the replacement of fine roots by trees. Not only does this process require large amounts of carbon from the tree, it also inputs large amounts of this carbon into forest floors. This large nutrient input is fundamental to carbon and nutrient cycling in forest ecosystems (Gill & Jackson 2000) and is described in more detail in Chapter 2.

As discarded fine root tissues are comprised largely of lignocellulose and can only be completely decomposed by saprophytic and pathogenic fungi, the different functional groups of root associated fungi may change during decomposition. This change will

likely be away from fungi that require a living host (mycorrhizal and endophytic) to those that do not depend on living hosts (saprotrophs and pathogens). However, there is a knowledge gap regarding the ways in which fine root associated fungal communities actually change during decomposition. Because fine roots represent vast quantities of carbon that are continuously entering the soil environment, an understanding of the fungi that break them down may provide valuable information regarding carbon and nutrient cycling in forests. The goal of this study was to characterise how fungal communities responds to fine root senescence in *Abies balsamea* and *Picea rubens*, two trees common in temperate and boreal forests.

Materials and Methods

Sample Collection

See Chapter 2 for details on site descriptions and harvesting procedures. Fine root systems were sampled for each tree species at each site and at each time interval (Table 1). Fine roots from five time intervals were used for DNA extraction. DNA extraction was limited to these time intervals to minimise cost while providing a temporal resolution adequate to observe changes in community structure.

DNA Extraction

Fine roots were thawed and randomly selected as described for the mantle classification in Chapter 2. Primary fine roots, the most distal roots in a root system (Goebel et al. 2011), were removed from secondary fine roots under dissecting microscope (Nikon model SMZ800, Nikon Inc., Tokyo, Japan) in distilled water with

metal tweezers and measured along a piece of dampened filter paper (Whatman No 2, GE Healthcare Life Sciences, Mississauga, ON).

Because the primary fine roots of *A. balsamea* and *P. rubens* differ physically (shorter and wider in *A. balsamea* and longer and narrower in *P. rubens*), a predetermined number of fine roots was used for DNA extraction (an equal number of *A. balsamea and P. rubens* fine roots would contain more *A. balsamea* tissue and more *A. balsamea* ECM DNA). An equivalent length of primary fine roots was taken from each species for DNA extraction. Fifty cm of primary fine roots from each species were dried at 60°C for 12 hours and weighed using an analytical balance (Denver Instruments PI-214, Bohemia, NY) resulting in 12.2 mg for *A. balsamea* and 8.8 mg for *P. rubens* (a ratio of approximately 1 to 1.5). Therefore, 2 cm of primary fine roots were harvested per *A. balsamea* sample and 3 cm per *P. rubens* sample. This relatively short length of tissue was chosen as root systems from later time intervals had a limited number of primary fine roots remaining after decomposition.

Primary fine roots were placed in a 2 ml O-ring tube with 500 mg of silica beads (Omni International, Kennesaw, GA) and 600 µl of AP1 buffer (DNeasy Plant Mini Kit, Qiagen, Hilden, Germany) then oscillated at 5000 RPM for 10 minutes in a Omni Bead Ruptor Homogenizer (Omni International, Kennesaw, GA). DNA extraction then followed protocol of the Qiagen DNeasy plant extraction kit (Qiagen, Hilden, Germany). All DNA extracts were diluted 1:10 in distilled water and stored at -20°C.

Polymerase Chain Reaction (PCR)

The internal transcribed spacer (ITS) region was amplified from each sample using the forward primer ITS1f (3′- TCCGTAGGTGAACCTGCGG-5′) (Gardes & Bruns 1993) and the reverse primer ITS4 (3′-TCCTCCGCTTATTGATATGC-5′) (White et al. 1990). ITS1f anneals in the 18S (SSU) rRNA gene, while ITS4 anneals in the 28S (LSU) rRNA gene. This amplifies the ITS1 region, the conserved intercalary 5.8S rRNA gene, and the ITS2 region. The ITS1 and ITS2 regions are commonly used as "barcoding" regions for fungi and multiple sequence databases are available (Schoch et al. 2012). These regions are highly divergent and vary at the species level.

Reactions were performed on a Veriti 96 Well Thermocycler (Applied Biosystems, Foster City, CA) in 25-µl reactions. Each 25-µl reaction contained: 12.5 µl GoTaq® Green Master Mix (Promega, Madison, WI), 2.5 µl ITS1f (2.5 µM) and 2.5 µl ITS4 (2.5 µM) (White et al. 1990) (IDT, Coralville, IA) and 7.5 µl of DNA. PCR amplification parameters were as follows: 94°C for 60 s, followed by 30 cycles of 94°C for 60 s, 60°C for 60 s, and 72°C for 120 s, with a final elongation at of 72°C for 10 m. Products were run on 1.5% agarose gels with 1X sodium-borate buffer using EZ-vision gel dye (Amresco®, Solon, OH) and an EC 105 electrophoresis power supply (E-C Apparatus Corporation, USA). Gels were imaged at 365 nm wavelength using an AlphaImager HP gel imaging system (ProteinSimple, San Jose, CA). Amplification was considered successful when products produced bands of approximately 1000 base pairs. Amplicons were stored at -20°C.

Molecular Cloning

Amplicon libraries were purified following the protocol of the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and ligated into vector plasmids according to pGEM®-T Easy Vector Systems protocol (Promega, Madison, WI). Ligated plasmids were transformed into competent NEB 5-alpha *E. coli* cells (New England Biolabs, Ipswich, MA). After the incubation period, competent cells were plated onto lysogeny broth medium and incubated at 37°C for 24 hours in a HeraTherm ICS100 incubator (Thermo Scientific, Waltham, MA). Twelve isolated colonies from each plate were transferred into 30 µl of distilled water using sterile toothpicks.

Colony PCR

The ligated fungal ITS was amplified from each *E. coli* culture using ITS1 and ITS4 (Gardes & Bruns 1993). The annealing site of the ITS1 is located downstream of the ITS1f site, affording a semi-nested PCR. Each 25-µl reaction contained: 12.5 µl GoTaq® Green Master Mix (Promega, Madison, WI), 2.5 µl ITS1 and 2.5 µl ITS4 (IDT, Coralville, IA) both at final concentrations of 2.5 µM, and 7.5 µl of DNA extract. PCR amplification parameters were the same as those reported above for preliminary-PCR. Products were run on gels as described above. Amplification was considered successful if products were approximately 1000 base pairs long. PCR products were sequenced using an ABI PRISM 3730XL DNA analyzer system using ITS1 and ITS4 primers at the McGill University and Genome Québec Innovation Centre.

ITS Sequence Analysis

Forward and reverse ITS sequences were aligned using Sequencher 5.3 (Gene Codes Corporation, Ann Arbor, MI) to manually correct base pair discrepancies and create consensus sequences. Each consensus was queried in GenBank (blast.ncbi.nlm.nih.gov) using BLAST (megablast – exact matches). Operational taxonomical unit (OTU) assignment was based on a combination of the highest match score and the validity of database entry (voucher samples were favoured over environmental samples). When sequence matches on NCBI were ectomycorrhizal, they were subsequently queried in the UNITE (unite.ut.ee) database, which specializes in ectomycorrhizal fungi (Kõljalg et al. 2005).

Sample Diversity, Analysis of Similarity, and Similarity Percentages

All computations of diversity, rank-abundance, and ordinations were completed using the "vegan" (Oksanen et al. 2005) and "BiodiversityR" (Kindt & Coe 2005) packages in R statistical software (R Core Team 2015). Response of fungal community to treatment was analyzed across sites and seedling species. The OTUs detected in each sample were categorized into genera as identification to species was not always possible (Appendix 2, Table 1). The resulting community table was square-root transformed and similarity between samples was quantified according to the Bray-Curtis dissimilarity. Analysis of similarities (ANOSIM) was used to determine which predictor variables caused significant differences in fungal communities. Similarity percentages (SIMPER) analysis was used to determine to what degree OTUs contributed to overall similarity and

dissimilarity between each community. ANOSIM and SIMPER tests (Clarke 1993) were performed using Primer-E (v. 6) software (Clarke & Gorley 2006).

Results

DNA Sequencing and OTU Assignment

DNA sequencing resulted in 317 unique OTUs; these sequences represented species from 33 different fungal genera (Table 3). Identification to genus was not possible for 34 sequences; therefore, these were assigned an OTU at the family level as further identification was not possible. For the 34 sequences that were only identifiable to the family level, 33 came from families within the order Helotiales, with the majority of these in the Helotiaceae.

Redundancy Analysis, Analysis of Similarity, and Similarity of Percentages

Redundancy analysis (RDA) showed that the main difference in fungal communities was driven by seedling species and that the Helotiaceae and *Piloderma* were highly influential (Figure 1). This was supported by analysis of similarity (ANOSIM) which showed the variation between fungal communities was being driven mostly by seedling species (Global R = 0.201, p = 0.008, permutations = 99,999). Similarity of percentages (SIMPER) analysis showed that Helotiaceae sp. accounted for the highest percentage of community similarity within *A. balsamea* while *Piloderma* was highest in *P. rubens* communities (Table 4). Regarding dissimilarity between seedling communities, *Piloderma* had the highest contribution to dissimilarity with Helotiaceae contributing second most (Table 5).

Ectomycorrhizal Fungi

Overall, ectomycorrhizal fungi were the most abundant functional group and made up the largest proportions of OTUs in most of the fungal communities (Figure 2 and Figure 3). The most common genera were *Piloderma, Cenococcum, Russula, Cortinarius,* and *Clavulina,* in decreasing abundance (Figure 4). The proportion of ECM genera changed over time: *Piloderma* showed an overall increase in treatment seedlings compared to controls and was dominant at every time interval except one; *Cenococcum* showed decreases in proportion compared to controls, but started to return to control levels by the final time interval; *Russula* increased in proportion compared to controls especially in the last and second last time intervals; *Cortinarius* showed a steady decrease in proportion compared to its levels in controls; and *Clavulina* showed an inconsistent presence between time intervals, and were not detected at the last time interval (Figure 4). Regarding host preference, *Piloderma* was detected twice as much in *P. rubens, Cenococcum* was more abundant in *A. balsamea,* and *Cortinarius* was almost exclusively detected in *P. rubens* (Table 5). *Russula* and *Clavulina* had nearly equal abundances in both seedling species (Table 5).

Endophytic Fungi

Endophytes were detected from the first time interval post treatment through to the last time interval (Figure 3). The most abundant endophytic fungi belonged to Helotiaceae sp. OTU and *Meliniomyces*. Both of these showed high host specificity with Helotiaceae sp. detected almost exclusively in *A. balsamea* and *Meliniomyces* showing preference for *P. rubens*. Regarding other endophytic species, *Acremonium, Chloridium,*

and *Oidiodendron* were detected only in *A. balsamea* whereas *Rhizoscyphus,* Hyaloscyphaceae sp., and Herpotrichellaceae sp. were detected only in *P. rubens* (Table 5).

Saprotrophic Fungi

Saprotrophic fungi were the least common functional group, appearing most at mid to late time intervals. One exception to this was *Galerina* sp. which appeared strongly in *A. balsamea*. *Galerina* sp. was the most commonly identified saprotroph and showed a preference for *A. balsamea* (Table 5). *Picea rubens* had a broader range of saprotrophic fungi identified including members of *Leotia, Cryptococcus,* and *Penicillium* (Table 5).

Discussion

Ectomycorrhizal fungi

We found that the proportion of clones representing ECM fungi did not decrease as expected during the course of the decomposition experiment, even though the ECM mantles clearly became degraded. There are a few reasons why the ECM signal may have persisted. First, the presence of ECM DNA in a fine root does not imply a functional, or even a living ectomycorrhiza. The extensive internal network of hyphae forming the Hartig net within primary fine roots may remain after the exterior mantle has died and began to decompose. Decomposition of ectomycorrhizae has been observed to begin in the outer mantle cells and progress inward (Downes et al. 1992) resulting in slower decomposition of internal fungal tissues. Also, some ECM types produce a large amount of extramatrical hyphae for nutrient acquisition (Agerer 2001) which may also persist

after the mantle tissue has degraded. For example, some species of *Piloderma,* which had a strong presence at later sampling times, produce extensive mats of extracellular hyphae. Sclerotia, resistant masses of compact mycelia used for asexual regeneration in extreme environments, may also harbour fungal DNA after the ECM has decomposed. *Cenococcum geophilum* produces large numbers of resistant sclerotia and was well represented toward the end of the decomposition experiment.

The presence of recalcitrant and antifeedant compounds may also explain why some species of ECM fungi remained into later stages of the study. Some species of *Piloderma* produce hyphae that are encrusted with calcium oxalate crystals; which may deter invertebrates thereby inhibiting decomposition. The genus *Russula,* the second most abundant genus of ECM at the final time interval, contains species that produce sesquiterpenes. These volatile organic compounds are thought to play a role in chemical defence and likely have antifeedant properties. Finally, pigmentation may also be a factor in ECM decomposition. *Cenococcum geophilum* is highly melanised and was still well represented in the cloning data 16 months post treatment. In contrast, members of *Cortinarius* were abundant in control seedlings, but showed a rapid decline in abundance post treatment. This may be due to a lack recalcitrant or antifeedant compounds.

The inconsistent appearance of *Clavulina* may be explained by the cloning technique only being able to capture a "snapshot" of the community; because only 12 cultures of *E. coli* were used for sequencing at each time interval, it may have been possible that *Clavulina* did not get included by chance. This being said, the same logic

holds true for the other common ECM fungi and they showed consistent appearance at each time interval.

Finally, some ECM fungi may have facultative saprotrophic capabilities, allowing them to digest their root cell walls when host carbon is no longer provided. Under normal circumstances, some ECM can decompose components of plant litter and transport the nutrients back to their host (Courty et al. 2007). Plant litter older than three years is usually devoid of cellulose and hemicellulose (Osono & Takeda 2006) but has high concentrations of recalcitrant compounds such as lignin. Some ECM fungi produce digestive enzymes including cellobiohydrolase, b-glucosidase, xylosidase, and glucouronidase, all of which depolymerise organic substrates into glucose. Further, certain ECM fungi can produce laccase, enzymes involved in the digestion of lignin (Hatakka 1994; Eggert et al. 1996) which could allow for the decomposition of recalcitrant plant litter. Lindahl et al. (2007) propose that ECM fungi use their access to host sugars to co-metabolise recalcitrant organic matter as it is a source of sequestered N (Rineau et al. 2012; Bodecker et al. 2014; Lindahl & Tunlid 2015). It seems therefore that certain ECM fungi may have the capacity to decompose their former host in times of resource scarcity such as after fine root tip senescence. This may be a partial explanation for the abundance of some ECM genera in the late stages of the study.

Multiple factors are believed to influence saprotrophic behaviour in ECM fungi. In laboratory experiments, carbon starved ECM fungi can produce cellulolytic and proteolytic enzymes (Courty et al. 2007; Baldrian 2009), a trait that may be useful when host carbon is no longer supplied in adequate amounts (Buée et al. 2005; Courty et al.

2007) and the ECM fungus needs to seek out alternative carbon sources (Lindahl & Tunlid 2015). Members of the genus *Piloderma* exhibit the ability to acquire carbon in the absence of a plant host (Erland et al. 1990). Hagerman et al. (1999) propose that their observations of living *Piloderma* on fine roots two years after a clear-cut event suggests saprotrophic activity on dying fine roots. Some species of *Piloderma* have been shown to possess laccase-like genes that are expressed during high nitrogen conditions (Chen et al. 2003). These saprotrophic abilities may explain why *Piloderma* was the most abundant ECM fungi after 16 months post treatment in our study.

Hagerman et al. (1999) also found that members of *Lactarius* formed higher proportions of ectomycorrhizae at clear-cut sites than *Cortinarius* compared to proportions found in forest. Some *Lactarius* have been shown to produce enzymes involved in the depolymerisation of lignin (Giltrap 1982) and these could possibly be sued to decompose organic matter for carbon and nutrients. In our study *Cortinarius* made up a high proportion of control samples but decreased steadily in post treatment samples over time. When a host stops providing carbon, whether it is due to host death or fine root turnover, fungi such as *Cortinarius* may be the first to disappear from the community for a number of reasons. First, they may lack or have relatively low saprotrophic ability compared to fungi like *Piloderma* and *Lactarius*, and be outcompeted. Second, their lack of antifeedant/recalcitrant secondary metabolites (melanin, calcium oxalate, sesquiterpenes, *etc*...) may cause them to decompose quickly upon death resulting in their tissues being removed quickly from the community.

There is debate regarding whether the properties listed above warrant some ECM to be classified as facultative saprotrophs (Baldrian 2009). Some researchers propose that instead of adhering to concrete functional classifications, it is more accurate to describe fungi along a biotrophic-saprotrophic continuum (Koide et al. 2008). Baldrian (2009) makes the point that as ECM fungi would still be dependent on their host for substrate, they could be described as decomposers but not saprotrophs. This argument seems semantic in nature as all saprotrophs can be thought of as dependent on plants as they produce the substrates used by saprotrophs to survive. It seems that the differences between decomposer and saprotroph are based on independence from a plant host (Baldrian 2009).

In summary, some ECM fungi may have the potential to decompose organic matter. *Piloderma* dominated the ECM community 16 months post-treatment and is thought to have saprotrophic ability. In contrast, *Cortinarius* were almost absent at this time. Whether or not the fungi detected in the last time interval are viable is difficult to determine; external fungal tissues may have been dead, but Hartig nets may have viable due to their internal location. Antifeedants and recalcitrant compounds may also play a role. More studies into these traits are needed before conclusions can be reached regarding their effect on survivability and decomposition.

Fungal root endophytes

The most abundant endophytic fungus in this study, Helotiaceae sp., was identifiable only to the family level (Helotiaceae). This endophyte shared 100% ITS1, 5.8S rRNA, and ITS2 sequence similarity with Helotiaceae sp. VI reported by Kernaghan

& Patriquin (2011). In their study of root endophytes in boreal trees (*Abies balsamea, Picea glauca,* and *Betula papyrifera*) they identified seven Helotialian endophytes which were assigned the operational names Helotiaceae I-VII. These endophytes are closely related to the *Rhizoscyphus ericae* aggregate based on a 96-98% similarity between their ITS1, 5.8S rRNA, and ITS2 sequences (Kernaghan & Patriquin 2011). Considering that geographically separated *R. ericae* show less than 3.5% sequence variance (Egger & Sigler 1993; Hambleton & Currah 1997), Helotiaceae sp. is likely closely related to *R. ericae.* Interestingly, *R. ericae* was detected in our study although only in *P. rubens* whereas Helotiaceae sp. was almost exclusively found in *A. balsamea.* Although *R. ericae* is an ericoid mycorrhizal (ERM) fungus known for its presence in the Ericaceae, various ERM have been reported in coniferous ECM (Bergero et al. 2000; Vrålstad et al. 2002; Collier & Bidartondo 2009).

As Helotiaceae sp. is the dominant endophyte in this study, but only identified to the family level, it is hard to make precise conclusions about its functionality. However, considering its high degree of similarity to *R. ericae*, the behaviours reported for *R. ericae* may also be indicative of the ecological function of Helotiaceae sp. Ericoid mycorrhizae are formed in plants belonging to the Ericaceae and are common in acidic, nutrient poor soils of Northern latitudes (Cairney & Burke 1998). Unlike ECM fungi, ERM do not produce mantles or large amounts of exploratory hyphae, instead they only extend a few millimetres into the rhizosphere. Because of this, it is believed that the major benefit that conferred by *R. ericae* and other ERM to their host is their ability to degrade sources of organic material in the soil and transfer back the resulting products

(Smith & Read 1997). In their review of the saprotrophic ability of ECM, Lindahl & Tunlid (2015) state that ERM show more saprotrophic ability than ECM. In laboratory experiments, *R. ericae* produces a range of enzymes involved in decomposition including cellulases, hemicellulases, polygalacturonase, and polyphenol oxidases (Cairney & Burke 1998 and references within), which may facilitate the decomposition of plant litter and the acquisition of N and P. *Rhizoscyphus ericae* also tolerates and detoxifies phenolic acids and tannin complexes (Leake & Read 1990; Leake & Read 1991), which are common in coniferous roots. Cairney & Burke (1998) admit that the enzyme expression in *R. ericae* is variable and that most of the work reported had been completed in a laboratory setting. However, their review strongly suggests that *R. ericae* plays a role in decomposing organic matter in *mor* humus (Green et al. 1993), a humus type typical of coniferous forests and are rich in fungi.

The increase in the proportion of DNA representing Helotiaceae sp. and other endophytic fungi post-treatment indicates that host senescence may cause some endophytes to increase in abundance relative to their levels in living seedlings. The increase could be indicative of endophytes acting as latent saprotrophs, utilising the readily accessible labile compounds (*i.e.* pectin) of their senescent host. However, this could also be interpreted as a final effort by the endophytes to produce exploratory tissues used to find other hosts. Without more detailed analysis of fine root biochemistry during this process, it is difficult to make conclusions regarding the increase in root endophytic fungi after plant death.

Saprotrophs

Saprotrophs did not make up large portions of the fungal communities; half of the saprotrophic OTUs were singletons. Most saprotrophs observed appeared at later time intervals, except for *Galerina* which appeared relatively early in *A. balsamea. Galerina* was four times more abundant than any other saprotrophic genus and was the dominant saprotroph found in *A. balsamea*. The saprotrophic community of *P. rubens* was more even, being made up of equal parts of *Leotia, Cryptococcus*, and *Penicillium.* The relatively low abundance of saprotrophs compared to ECM and endophytic fungi implies that they are not responsible for the majority of the decomposition that occurs within the first 16 months of fine root senescence. This is not surprising considering the relatively slow decomposition of organic materials in northern coniferous forests.

It is interesting that the abundance of *Galerina* increased concurrently with the endophytes. Although *Galerina* is typified as being a saprotroph, it has been suggested that some members of this genus may have biotrophic or endophytic stages in mosses (Gulden 2008). OTUs identified as *Galerina* appeared both in dead and living portions of moss tissue signifying that it may be adapted to more than just a saprotrophic niche (Heimdal 2012). Whether or not *Galerina* could have a similar relationship with conifers is yet to be tested, but it could explain why this genus was both the most common and the earliest saprotroph detected in our study. Alternatively, as many of the plots in this study had significant moss cover, *Galerina* may have been the dominant saprotroph in the soil community which resulted in its abundance and early detection. This being said, it is odd

that *Galerina* was almost exclusively isolated from *A. balsamea,* as host preference is more typical of a biotrophic or endophytic relationship than a saprotrophic one.

The slow onset of saprotrophic fungi relative to other functional groups implies that saprotrophic decomposition of fine roots in the Acadian Forest does not occur appreciably during the first 16 months after root death. Although this agrees with other descriptions of litter decomposition in northern forests, the saprotrophic diversity may be underestimated as a result of functional classification. As in endophytes, whether or not certain fungi should classified as saprotrophs is not always clear. Because of this, there may be more saprotrophic decomposition of fine roots than is made apparent by simply characterising changes in the functional groups of the fungal community. Biochemical quantification of root constituents and mRNA analyses during fine root decomposition may shed light on decomposition in fine roots, as there will always be debate as to which species belong to which functional groups. For instance, as with *Galerina,* there is evidence suggesting that *Mycena* may have endophytic stages (Kernaghan & Patriquin 2011) and that *Geoglossum* is also not purely saprotrophic (Ohenoja et al. 2010). While the assignment of genera like *Penicillium, Mortierella*, *Cryptococcus, Perenniporia*, and *Leotia* to the saprotroph category is fairly well supported, this proves difficult for under-characterised fungi like *Hannaella*. Saprotrophy seems to be the default functional group to which fungi are assigned in the absence of detailed information. This is understandable as many fungi act as decomposers, but it makes detailed ecological conclusions difficult and ultimately reflects the amount of work required for a better understanding of the fungal ecology involved in fine root decomposition.

Conclusion

The sequence data shows that ECM fungi dominate the fine root communities of *A. balsamea* and *P. rubens* both before and after treatment with a temporary increase in endophytic fungi. Although still dominant, the ECM community changes from a diverse assemblage of genera, to mainly *Piloderma* and *Russula.* The ECM at the end of the study could be: a) dead, but recalcitrant to decomposition because of secondary metabolites, melanisation, and antifeedants or b) living and receiving carbon in a saprotrophic manner either from their deceased host or from organic matter in the soil. The behaviour of ECM after host senescence is critical to better understanding and more accurately predicting carbon models for forests dominated by trees involved in ECM symbioses. Although this study investigated the changes root systems that were completely senescent, only parts of the root system actually become senescent during normal fine root turnover and ECM fungi may not behave similarly during both processes. The extent of ECM decomposition of fine root material in northern forests needs to be further researched.

Although plagued with semantic and classification issues, endophytes showed an initial increase in abundance following host treatment that began to subside in the later stages of the study. Whether this surge represents increased production from newly available host resources or an effort by the endophytes to find a new host is unknown, but warrants further study. As some root endophytes show the ability to produce enzymes used in decomposition, their effect on carbon cycling in forests could be significant as they are ubiquitous in root tissues (Mayerhofer et al. 2013b). Additionally, the

differences observed in endophytic communities between *A. balsamea* and *P. rubens* warrants further study. First, *A. balsamea* had almost twice as many endophytic OTUs than *P. rubens.* Secondly, the endophytic communities of *A. balsamea* were dominated by Helotiaceae sp. and *Oidiodendron* and *P. rubens* was dominated by *Meliniomyces* and *Rhizoscyphus*. These fungi are closely related and more information on their preference for *A. balsamea* should prove interesting. However, the problem of classifying fungi by functional groups makes it difficult to compare communities between these two seedling species with confidence.

Future Research

The sample size of sequence data was a major limitation of this study. Although cloning represented many hours of work, the data returned are relatively small (319 OTUs) compared to the amount returned by a next generation sequencing (NGS) platform, which can generate hundreds of thousands to millions of OTUs. Although a single run on a NGS platform is relatively expensive, analyses that require temporal resolution or large amounts of replication quickly become more expensive using traditional cloning methods. For future studies, it may be beneficial to do initial exploration of fungal communities using an NGS platform and following up with targeted cloning of communities during stages of interest.

Due to the labour intensity of molecular cloning, only controls at the beginning of the experiment had their fungal communities analysed. In future studies treatment seedlings should be analysed with control seedlings for each time interval. This could

capture seasonal fluctuations in fungal communities, allowing seasonal variation to be accounted for in future research.

As was shown by the slow mobility of saprotrophic fungi, decomposition of fine roots would benefit from a longer observational period. This may be difficult to achieve within the duration of a typical graduate degree. An alternative would be to analyse fungal communities of fine roots at sites where host death is both known and staggered (*i.e.* a nursery), allowing for the fungal communities over time to be analysed without having to wait for the time to pass. This is often problematic as root systems are sensitive to anthropogenic disturbances and the amount of information known about a certain plot of forest is usually positively correlated with disturbances to that site. A compromise needs to be developed where decomposition of fine roots in a relatively undisturbed forest ecosystem can be monitored without traumatic disturbances to the soil ecosystem. This may be more achievable in the future as remote sensory technologies (minirhizotrons) become more affordable. *In lieu* of technological advancement, fungal communities of fine roots at clear-cuts could be studied in further detail (*e.g.* Hagerman et al. 1999). Additionally, tree nurseries could be studied as harvest date would be known and terrain would be less disturbed. Depending on the harvesting schedule of the nursery, decomposition at various times could be measured at the same location. Finally, plots of forest that have experienced selective harvest may prove the best compromise; these plots would have soil communities more typical of undisturbed forest and would not suffer disturbances as severe as the forest floor does during clear-cuts.

An aspect of the project design worth noting is that all primary roots analysed needed to be attached to secondary fine roots of the root system after root systems were extracted from the soil. In addition to the many fine roots lost during the extraction procedure, primary fine roots that were discarded into the rhizosphere were not able to be sampled. Although we speculate the communities are likely similar, any differences between communities decomposing intact root systems compared to those only in the primary fine roots was not touched upon by this study. Studies looking at the communities of fine roots lost during fine root turnover would be desirable but collecting these tissues without severely disturbing their environment is currently not achievable.

Finally, to better understand fungal decomposition of fine roots, the constituents of fine roots should be measured over the decomposition process. Levels of labile compounds such as sugars and proteins could be identified using biochemical tests or an HPLC. The amount of fungal tissue in fine roots may also be quantified using a similar method. Although there are many litter decomposition studies in the literature, to the knowledge of the author, none combine the quantification and description of decomposition of fine roots with changes in their fungal communities. This may shed light on whether some groups of fungi are filling saprotrophic niches by decomposing certain components of the fine roots, and would further elucidate the process of fine root decomposition.

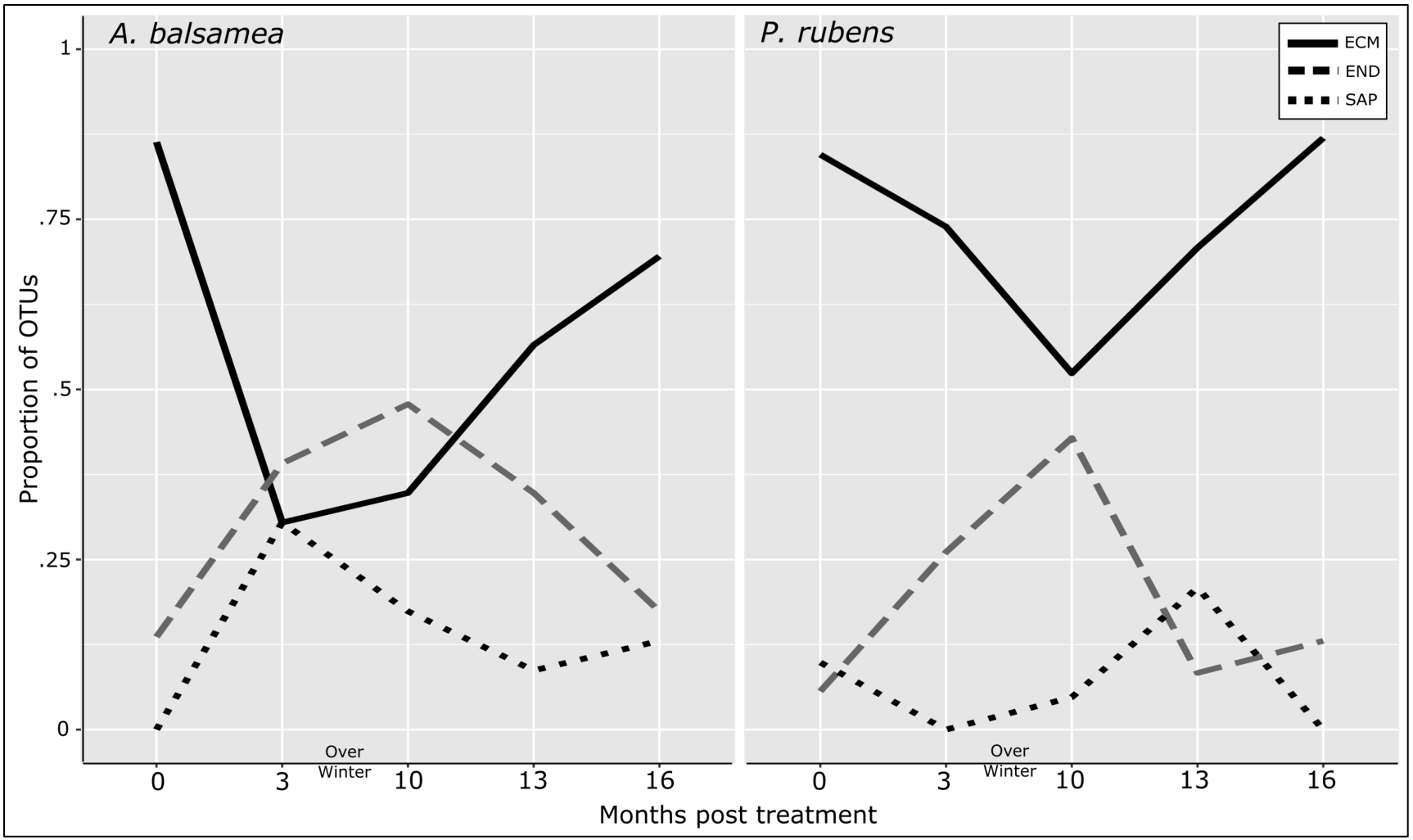

Figure 1. Proportion of OTUs made up by the different functional groups of root associated fungi up to 16 months post-treatment.

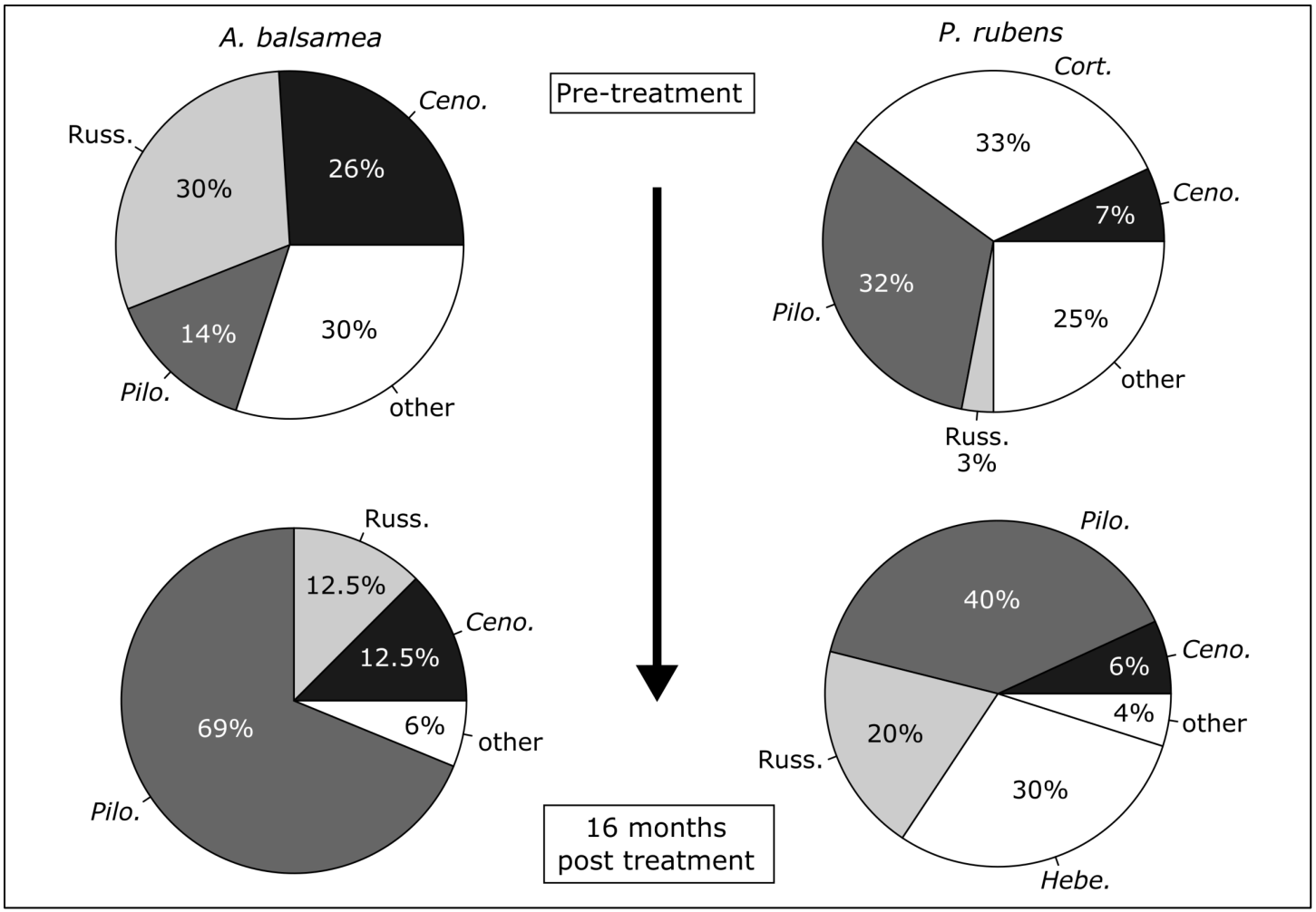

Figure 2. Ectomycorrhizal community (proportion of OTUs) in pre-treatment and 16 month post-treatment seedlings.

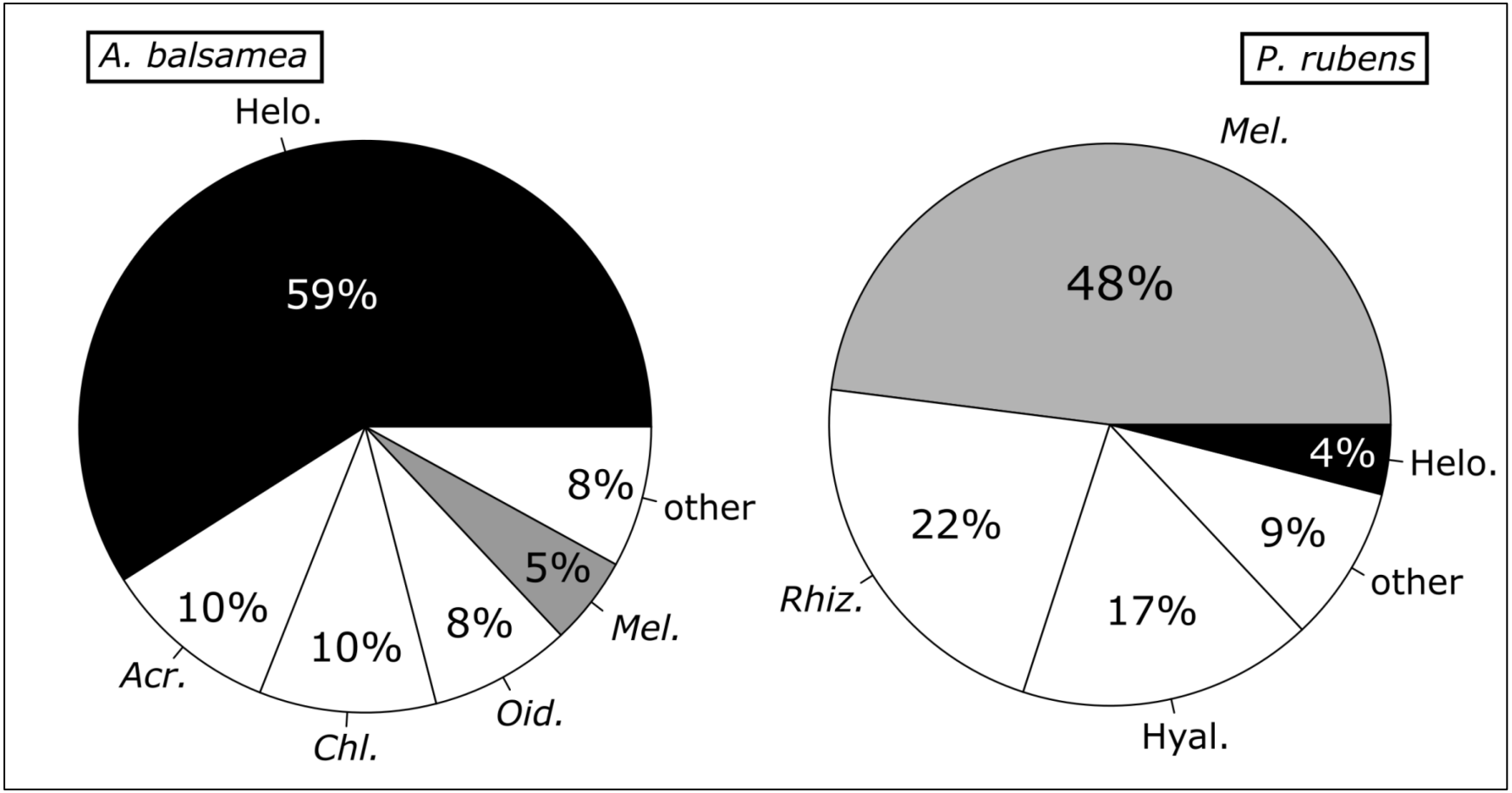

Figure 3. Endophytic community (proportion of OTUs) in post-treatment seedlings.

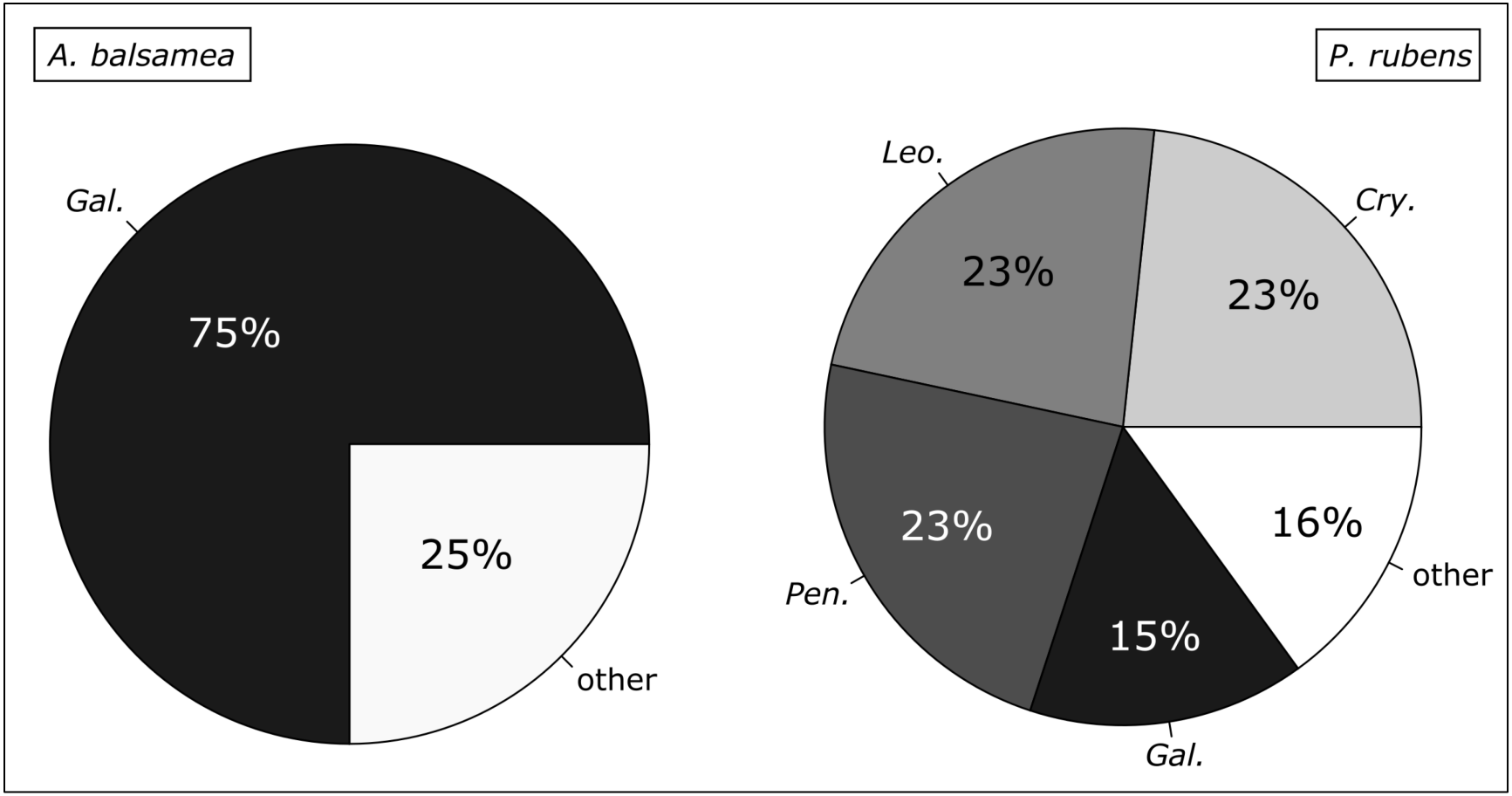

Figure 4. Saprotrophic community (proportion of OTUs) in post-treatment seedlings.

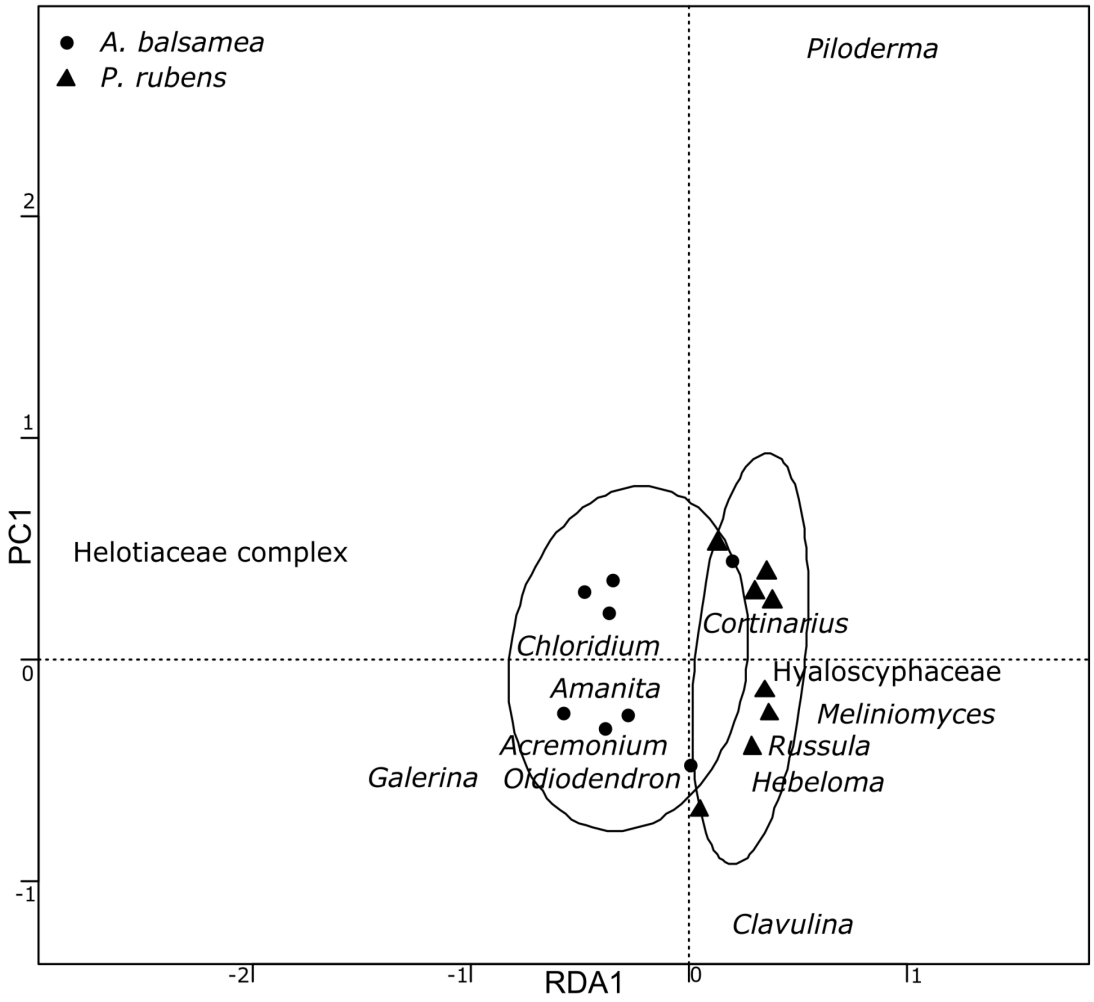

Figure 5. Redundancy analysis (RDA) for treatment seedlings from both *A. balsamea* and *P. rubens* across all times. Ellipses represent 95% confidence intervals around centroid. Results based on 9,999 permutations.

Table 1. List of literature supporting categorization of OTUs into functional groups (FG).

OTU	FG	References
Acremonium	END	Siegel 1993
Amanita	ECM	Mleczko 2004; Hobbie et al. 2001; Bidartondo et al. 2000; Högberg et al. 1999; Cripps & Miller 1995
Camarophyllopsis	ECM	Birkebak 2013
Cenococcum	ECM	Mahmood et al. 1999; Harniman & Durall 1996; Godbout & Fortin 1983; Trappe 1962
Chloridium	END	Harney et al. 1997; Wilcox & Wang 1985; Wilcox et al. 1974; Hammill 1972
Clavulina	ECM	Tedersoo et al. 2013; Tedersoo & Smith 2013; Matheny et al. 2009; Tedersoo et al. 2003
Clavulinopsis	SAP	Brundrett et al. 1996
Cortinarius	ECM	Mleczko 2004; Kuss et al. 2004; Hobbie et al. 2001; Högberg et al. 1999; Godbout & Fortin 1983
Cryptococcus	SAP	Botha 2011; Botha 2006
Elaphomyces	ECM	Tedersoo et al. 2003; Agerer 1999; Miller & Miller 1984
Galerina	SAP	Mleczko 2004; Hobbie et al. 2001
Geoglossum	SAP	Molina et al. 1992
Hannaella	SAP	Landell et al. 2014; Botha 2011
Hebeloma	ECM	Clemmensen et al. 2006; Mleczko 2004; Jakucs et al. 1999; Högberg et al. 1999; Brunner et al. 1991; Trappe 1962
Helotiaceae sp.	END	Kernaghan & Patriquin 2011
Hyphodiscus	SAP	Ottoson et al. 2015; Tedersoo et al. 2009
Inocybe	ECM	Mleczko 2004; Hobbie et al. 2001; Högberg et al. 1999; Magyar et al. 1999; Cripps & Miller 1995
Lactarius	ECM	Flores et al. 2005; Nuytinck et al. 2004; Eberhardt et al. 2000; Hobbie et al. 2001; Högberg et al. 1999
Leotia	SAP	Molina et al. 1992
Meliniomyces	ECM	Twieg et al. 2007; Brand et al. 1992
Mortierella	SAP	Kernaghan 2013; Bååth & Söderström 1980
Mycena	SAP	Hobbie et al. 2001; Högberg et al. 1999
Oidiodendron	END	Rice & Currah 2006; Lacourt et al. 2000; Couture et al. 1983
Penicillium	SAP	Kernaghan 2013; Bååth & Söderström 1980; Siu 1951
Perenniporia	SAP	Ben-Younes et al. 2007
Phialocephala	END	Menkis et al. 2005; Harney et al. 1997; O'Dell et al. 1993; Wang & Wilcox 1985
Piloderma	ECM	Baxter & Dighton 2001; Dahlberg et al. 1997; Goodman & Trofymow 1996; Molina et al. 1992
Russula	ECM	Hobbie et al. 2001; Beenken 2001; Högberg et al. 1999; Taylor & Alexander 1989; Trappe 1962

Rhizoscyphus	END	Grelet et al. 2010; Zhang & Zhuang 2004; Cairney & Burke 1998; Kernan & Finocchio 1983
Sistotrema	ECM	Di Marino et al. 2008; Nilsson et al. 2006
Tomentella	ECM	Agerer 2006; Jakucs et al. 2005; Kõljalg 1992
Trechispora	ECM	Dunham et al. 2007
Truncocolumella	ECM	Horton et al. 2005; Massicotte et al. 2000; Eberhardt & Luoma 1996; Trappe 1962
Tylospora	ECM	Agerer 2006; Eberhardt et al. 1999; Taylor & Alexander 1991

Table 2. OTUs from control and treatment seedlings across all time intervals for *A. balsamea* and *P. rubens.*

OTU	Abun.	*A. balsamea*	*P. rubens*	Prop. (%)	Cum.(%)	FG
Piloderma	67	23	44	21.0	21.0	ECM
Cenococcum	29	20	9	9.1	30.1	ECM
Russula	28	16	12	8.8	38.9	ECM
Cortinarius	25	2	23	7.8	46.7	ECM
Helotiaceae	24	23	1	7.5	54.2	END
Clavulina	21	10	11	6.6	60.8	ECM
Galerina	14	12	2	4.4	65.2	SAP
Lactarius	13	13	0	4.1	69.3	ECM
Meliniomyces	13	2	11	4.1	73.4	END
Trechispora	10	2	8	3.1	76.5	ECM
Hebeloma	7	0	7	2.2	78.7	ECM
Truncocolumella	6	6	0	1.9	80.6	ECM
Rhizoscyphus	6	1	5	1.9	82.5	END
Tomentella	5	0	5	1.6	84.1	ECM
Amanita	4	4	0	1.3	85.4	ECM
Sistotrema	4	4	0	1.3	86.7	ECM
Acremonium	4	4	0	1.3	88.0	END
Chloridium	4	4	0	1.3	89.3	END
Hyaloscyphaceae sp.	4	0	4	1.3	90.6	END
Tylospora	3	0	3	0.9	91.5	ECM
Oidiodendron	3	3	0	0.9	92.4	END
Cryptococcus	3	0	3	0.9	93.3	SAP
Leotia	3	0	3	0.9	94.2	SAP
Penicillium	3	0	3	0.9	95.1	SAP
Camarophyllopsis	2	0	2	0.6	95.7	ECM
Herpotrichellaceae sp.	2	0	2	0.6	96.3	END
Dermateaceae	2	2	0	0.6	96.9	END
Elaphomyces	1	1	0	0.3	97.2	ECM
Inocybe	1	0	1	0.3	97.5	ECM
Phialocephala	1	1	0	0.3	97.8	END
Geoglossum	1	1	0	0.3	98.1	SAP
Hannaella	1	1	0	0.3	98.4	SAP
Mortierella	1	0	1	0.3	98.7	SAP
Mycena	1	1	0	0.3	99.0	SAP
Perenniporia	1	1	0	0.3	99.3	SAP
Ramariopsis	1	0	1	0.3	99.6	SAP
Mytilinidaceae	1	1	0	0.3	100.0	END

Table 3. SIMPER scores for OTU similarity between *A. balsamea* and *P. rubens*.

A. balsamea				
OTU	**Avg. abun.**	**Avg. sim**	**Cont. (%)**	**Cum. (%)**
Helotiaceae sp.	0.93	7.34	36.46	36.46
Piloderma	0.79	3.18	15.8	52.25
Cenococcum	0.71	3.09	15.35	67.61
Galerina	0.46	2.29	11.37	78.98
Russula	0.59	2.12	10.54	89.52
Lactarius	0.5	1.46	7.27	96.79

P. rubens				
OTU	**Avg. abun.**	**Avg. sim**	**Cont. (%)**	**Cum. (%)**
Piloderma	1.36	9.22	34.08	34.08
Russula	0.68	5.52	20.4	54.49
Cortinarius	0.77	3.76	13.89	68.37
Meliniomyces	0.49	2.42	8.94	77.31
Cenococcum	0.46	2.02	7.47	84.78
Hyaloscyphaceae sp.	0.29	1.87	6.9	91.68

Table 4. SIMPER scores for dissimilarity.

OTU	*A.balsamea*	*P.rubens*	Dissimilarity	Cont. (%)	Cum. (%)
Piloderma	0.79	1.36	9.83	12.01	12.01
Helotiaceae sp.	0.93	0.07	6.83	8.34	20.36
Cenococcum	0.71	0.46	6.43	7.86	28.22
Cortinarius	0.14	0.77	5.92	7.23	35.45
Russula	0.59	0.68	5.60	6.84	42.28
Clavulina	0.32	0.46	4.76	5.81	48.10
Meliniomyces	0.10	0.49	4.08	4.99	53.09
Galerina	0.46	0.10	3.73	4.55	57.64
Lactarius	0.50	0.00	3.53	4.31	61.95
Trechispora	0.10	0.34	2.88	3.52	65.47
Rhizoscyphus	0.07	0.27	2.42	2.96	68.43
Hyaloscyphaceae sp.	0.00	0.29	2.19	2.67	71.11
Hebeloma	0.00	0.25	1.98	2.42	73.53
Chloridium	0.24	0.00	1.86	2.27	75.79
Acremonium	0.24	0.00	1.78	2.17	77.96
Tomentella	0.00	0.21	1.48	1.80	79.77
Amanita	0.20	0.00	1.47	1.79	81.56
Sistotrema	0.20	0.00	1.47	1.79	83.35
Penicillium	0.00	0.17	1.26	1.54	84.89
Cryptococcus	0.00	0.17	1.24	1.52	86.41
Truncocolumella	0.17	0.00	1.16	1.42	87.82
Leotia	0.00	0.12	1.04	1.27	89.10
Herpotrichellaceae sp.	0.00	0.14	1.00	1.22	90.32

References

Agerer, R. (1999). *Elaphomyces aculeatus* Tul.+ *Quercus robur* L. *Descr. Ectomyc.* **4**: 37-41.

Agerer, R. (2001). Exploration types of ectomycorrhizae. *Mycorrhiza* 11:107-114.

Agerer, R. (2006). Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress* **5**:67-107.

Bååth, E., & Söderström, B. (1980). Degradation of macromolecules by microfungi isolated from different podzolic soil horizons. *Canadian Journal of Botany* **58**:422-425.

Baddeley, J. A., & Watson, C. A. (2005). Influences of root diameter, tree age, soil depth and season on fine root survivorship in *Prunus avium*. *Plant and Soil* **276**:15-22.

Baxter, J. W., & Dighton, J. (2001). Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (*Betula populifolia*) seedlings in host–symbiont culture conditions. *New Phytol*. **152**:139-149.

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.

Beenken, L. (2001). Russula alnetorum Romagn.+ Alnus viridis (Chaix) DC. *Descriptions of Ectomycorrhizae* **5**:115-123.

Ben-Younes, S., Mechichi, T., & Sayadi, S. (2007). Purification and characterization of the laccase secreted by the white rot fungus *Perenniporia tephropora* and its role

in the decolourization of synthetic dyes. *Journal of Applied Microbiology* **102**:1033-1042.

Bergero, R., Perotto, S., Girlanda, M., Vidano, G., & Luppi, A. M. (2000). Ericoid mycorrhizal fungi are common root associates of a Mediterranean ectomycorrhizal plant (Quercus ilex). *Molecular Ecology* **9**:1639-1649.

Bidartondo, M. I., Kretzer, A. M., Pine, E. M., & Bruns, T. D. (2000). High root concentration and uneven ectomycorrhizal diversity near Sarcodes sanguinea (Ericaceae): a cheater that stimulates its victims? *American Journal of Botany* **87**:1783-1788.

Birkebak, J. M., Mayor, J. R., Ryberg, K. M., & Matheny, P. B. (2013). A systematic, morphological and ecological overview of the Clavariaceae (Agaricales). *Mycologia* **105**:896-911.

Bödeker, I., Clemmensen, K. E., Boer, W., Martin, F., Olson, Å., & Lindahl, B. D. (2014). Ectomycorrhizal Cortinarius species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytol.* **203**:245-256.

Botha, A. (2006). Yeast in soil. In: Rosa, C.A., Péter, G. (Eds.) *The Yeast Handbook; Biodiversity and Ecophysiology of Yeasts.* Springer-Verlag: Berlin-Heidelberg.

Botha, A. (2011). The importance and ecology of yeasts in soil. *Soil Biology and Biochemistry* **43**:1-8

Brand, F. (1992). Mixed associations of fungi in ectomycorrhizal roots. *Mycorrhizas in Ecosystems* (pp 142-147). CAB International: Wallingford.

Brundrett, M. C. (2002). Coevolution of roots and mycorrhizas of land plants. *New phytol.* **154**:275-304.

Brundrett, M., Bougher, N., Dell, B., Grove, T., & Malajczuk, N. (1996). *Working with Mycorrhizas in Forestry and Agriculture: Australian Centre for International Agricultural Research*. Canberra.

Brunner, I., Amiet, R., & Schneider, B. (1991). Characterization of naturally grown and in vitro synthesized ectomycorrhizas of Hebeloma crustuliniforme and Picea abies. *Mycological Research* **95**:1407-1413.

Buée, M., Vairelles, D., & Garbaye, J. (2005). Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (Fagus silvatica) forest subjected to two thinning regimes. *Mycorrhiza* **15**:235-245.

Buée, M., De Boer, W., Martin, F., Van Overbeek, L., & Jurkevitch, E. (2009). The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. *Plant and Soil* **321**:189-212.

Cairney, J. W. G., & Burke, R. M. (1998). Extracellular enzyme activities of the ericoid mycorrhizal endophyte Hymenoscyphus ericae (Read) Korf & Kernan: their likely roles in decomposition of dead plant tissue in soil. *Plant and Soil* **205**:181-192.

Chen, D. M., Bastias, B. A., Taylor, A. F., & Cairney, J. W. (2003). Identification of laccase-like genes in ectomycorrhizal basidiomycetes and transcriptional regulation by nitrogen in Piloderma byssinum. *New Phytol.* **157**:547-554.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**:117-143.

Clarke, K. R., & Gorley, R. N. (2006). *PRIMER v6: User Manual/Tutorial. PRIMER-E.* Plymouth, UK.

Clemmensen, K. E., Michelsen, A., Jonasson, S., & Shaver, G. R. (2006). Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytol.* **171**:391-404.

Collier, F. A., & Bidartondo, M. I. (2009). Waiting for fungi: the ectomycorrhizal invasion of lowland heathlands. *Journal of Ecology* **97**:950-963.

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.

Cripps, C. L., & Miller Jr, O. K. (1995). Ectomycorrhizae formed in vitro by quaking aspen: including Inocybe lacera and Amanita pantherina. *Mycorrhiza* **5**:357-370.

Dahlberg, A., Jonsson, L., & Nylund, J. E. (1997). Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth

Norway spruce forest in south Sweden. *Canadian Journal of Botany* **75**:1323-1335.

Di Marino, E., Scattolin, L., Bodensteiner, P., & Agerer, R. (2008). Sistotrema is a genus with ectomycorrhizal species− confirmation of what sequence studies already suggested. *Mycological Progress* **7**:169-176.

Dighton, J. (2003). *Fungi in ecosystem processes*. CRC Press: Florida

Downes, G. M., Alexander, I. J., & Cairney, J. W. G. (1992). A study of ageing of spruce [Picea sitchensis (Bong.) Carr.] ectomycorrhizas. I. Morphological and cellular changes in mycorrhizas formed by Tylospora fibrillosa (Burt.) Donk and Paxillus involutus (Batsch. ex Fr.) Fr. *New Phytol.* **122**:141-152.

Drehmel, D., Moncalvo, J.-M., & Vilgalys, R.. (1999). Molecular Phylogeny of Amanita Based on Large-Subunit Ribosomal DNA Sequences: Implications for Taxonomy and Character Evolution. *Mycologia* **91**:610–618.

Dunham, S. M., Larsson, K. H., & Spatafora, J. W. (2007). Species richness and community composition of mat-forming ectomycorrhizal fungi in old-and second-growth Douglas-fir forests of the HJ Andrews Experimental Forest, Oregon, USA. *Mycorrhiza* **17**:633-645.

Eberhardt, U., Oberwinkler, F., Verbeken, A., Rinaldi, A. C., Pacioni, G., & Comandini, O. (2000). Lactarius ectomycorrhizae on Abies alba: morphological description, molecular characterization, and taxonomic remarks. *Mycologia* **92**:860-873.

Eberhardt, U., Walter, L., & Kottke, I. (1999). Molecular and morphological discrimination between Tylospora fibrillosa and Tylospora asterophora mycorrhizae. *Canadian Journal of Botany* **77**:11-21.

Eberhart, J., & Luoma, D. (1996). Truncocolumella citrina Zeller+ Pseudotsuga menziesii (Mirb.) Franco. *Concise descriptions of North American Ectomycorrhizae.* Mycologue Publications and Canada-BC Forest Resource Development Agreement. Canadian Forest Service, Victoria, BC, pp CDE9.

Egger, K. N., & Sigler, L. (1993). Relatedness of the ericoid endophytes Scytalidium vaccinii and Hymenoscyphus ericae inferred from analysis of ribosomal DNA. *Mycologia* **85**:219-230.

Eggert, C., Temp, U., & Eriksson, K. E. (1996). The ligninolytic system of the white rot fungus Pycnoporus cinnabarinus: purification and characterization of the laccase. *Applied and Environmental Microbiology* **62**:1151-1158.

Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., Robeson, M., Edwards, R. A., Felts, B., Rayhawk, S., Knight, R., Rohwer, F., & Jackson, R. B. (2007). Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Applied and environmental microbiology* **73**:7059-7066.

Finlay, R., & Söderström, B. (1992). *Mycorrhiza and carbon flow to the soil. Mycorrhizal functioning: An integrative plant-fungal process.* Lund University Publications.

Flores, R., Díaz, G., & Honrubia, M. (2005). Mycorrhizal synthesis of Lactarius indigo (Schw.) Fr. with five Neotropical pine species. *Mycorrhiza* **15**:563-570.

Fogel, R., & Hunt, G. (1983). Contribution of mycorrhizae and soil fungi to nutrient cycling in a Douglas-fir ecosystem. *Canadian Journal of Forest Research* **13**:219-232.

Couture, M., Fortin, J. A., & Dalpe, Y. (1983). Oidiodendron griseum Robak: An endophyte of ericoid mycorrhiza in Vaccinium spp. *New Phytol.* **95**:375-380.

Gallaud, T. (1905). Etudes sur les mycorrhizes endotrophes. *Revue Générale de Botanique* **17**:4-500.

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.

Gill, R. A., & Jackson, R. B. (2000). Global patterns of root turnover for terrestrial ecosystems. *New Phytol.* **147**:13-31.

Giltrap, N. J. (1982). Production of polyphenol oxidases by ectomycorrhizal fungi with special reference to Lactarius spp. *Transactions of the British Mycological Society* **78**:75-81.

Goebel, M., Hobbie, S. E., Bulaj, B., Zadworny, M., Archibald, D. D., Oleksyn, J., Reich, P. B., & Eissenstat, D. M. (2011). Decomposition of the finest root branching orders: linking belowground dynamics to fine-root function and structure. *Ecological Monographs* **81**:89-102.

Godbout, C., & Fortin, J. A. (1983). Morphological features of synthesized ectomycorrhizae of Alnus crispa and A. rugosa. *New Phytol.* **94**:249-262.

Goodman, D. M., & Trofymow, J. A. (1996). Piloderma fallax (Libert) Stalpers and Pseudotsuga menziesii (Mirb.) Franco. *A Manual of Concise Descriptions of North American Ectomycorrhizae.* Mycologue Publications, Victoria, BC.

Gulden, G. (2008). Galerina earle. In H. Knudsen & J Vesterholt (Eds.), *Funga Nordica: Agaricoid, Boletoid, and Cyphelloid Genera* (pp. 785-804). Nordsvamp: Copenhagen.

Green, R. N., Trowbridge, R. L., & Klinka, K. (1993). Towards a taxonomic classification of humus forms. *Forest Science* **39**:1-48.

Grelet, G. A., Johnson, D., Vrålstad, T., Alexander, I. J., & Anderson, I. C. (2010). New insights into the mycorrhizal Rhizoscyphus ericae aggregate: spatial structure and co-colonization of ectomycorrhizal and ericoid roots. *New Phytol.* **188**:210-222.

Grünig, C. R., Queloz, V., Sieber, T. N., & Holdenrieder, O. (2008). Dark septate endophytes (DSE) of the Phialocephala fortinii sl-Acephala applanata species complex in tree roots: classification, population biology, and ecology. *Botany* **86**:1355-1369.

Hagerman, S. M., Jones, M. D., Bradfield, G. E., Gillespie, M., & Durall, D. M. (1999). Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. *Canadian Journal of Forest Research* **29**:124-134.

Hammill, T. M.. (1972). Electron Microscopy of Conidiogenesis in Chloridium chlamydosporis. *Mycologia* **64**:1054–1065.

Hambleton, S., & Currah, R. S. (1997). Fungal endophytes from the roots of alpine and boreal Ericaceae. *Canadian Journal of Botany* **75**:1570-1581.

Harney, S. K., Rogers, S. O., & Wang, C. J. K. (1997). Molecular characterization of dematiaceous root endophytes. *Mycological Research* **101**:1397-1404.

Harniman, S. M. K., & Durall, D. M. (1996). Cenococcum geophilum Fr.+ Picea engelmannii (Parry) Engelm. Concise descriptions of North American Ectomycorrhizae. *Mycologue Publications and Canada-BC Forest Resource Development Agreement*, *Canadian Forest Service*, Victoria, BC, pp CDE10.

Hatakka, A. (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiology Reviews* **13**:125-135.

Hendrick, R. L., & Pregitzer, K. S. (1992). The demography of fine roots in a northern Deciduous forest. *Ecology* **73**:1094-1104.

Heimdal, R. S. (2012). *Using 454-sequencing for exploring diversity, host specificity and tissue specificity of the fungal genus* Galerina *associated with four boreal mosses* (Masters dissertation). Retrieved from University of Oslo (duo.uio.no).

Hildebrandt, U., Regvar, M., & Bothe, H. (2007). Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry* **68**:139-146.

Hobbie, E. A., Weber, N. S., & Trappe, J. M. (2001). Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. *New Phytol.* **150**:601-610.

Hobbie, E. A., Weber, N. S., Trappe, J. M., & Van Klinken, G. J. (2002). Using radiocarbon to determine the mycorrhizal status of fungi. *New Phytol.* **156**:129-136.

Högberg, P., Plamboeck, A. H., Taylor, A. F., & Fransson, P. M. (1999). Natural 13C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences* **96**:8534-8539.

Horton, T. R., Molina, R., & Hood, K. (2005). Douglas-fir ectomycorrhizae in 40-and 400-year-old stands: mycobiont availability to late successional western hemlock. *Mycorrhiza* **15**:393-403.

Jackson, R., Mooney, H. A., & Schulze, E. D. (1997). A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences* **94**:7362-7366.

Jakucs, E., Magyar, L., & Beenken, L. (1999). Hebeloma ammophilum Bohus+ Fumana procumbens (Dun.) Gr. Godr. *Descriptions of Ectomycorrhizae* **4**:49-54.

Jumpponen, A. R. I., & Trappe, J. M. (1998). Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytol.* **140**:295-310.

Jumpponen, A., & Jones, K. L. (2009). Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate Quercus macrocarpa phyllosphere. *New Phytol*. **184**:438-448.

Kernaghan, G. (2013). Functional diversity and resource partitioning in fungi associated with the fine feeder roots of forest trees. *Symbiosis* **61**:113-123.

Kernaghan, G., & Patriquin, G. (2011). Host associations between fungal root endophytes and boreal trees. *Microbial Ecology* **62**:460-473.

Kernan, M. J., & Finocchio, A. F. (1983). A new discomycete associated with the roots of Monotropa uniflora (Ericaceae). *Mycologia* **75**:916-920.

Kindt, R. & Coe, R. (2005) Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF): Nairobi.

Köljalg, U. (1992). Mycorrhiza formed by basidiospores of Tomentella crinalis on Pinus sylvestris. *Mycological Research* **96**:215-220.

Kõljalg, U., Larsson, K. H., Abarenkov, K., Nilsson, R. H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F., Tedersoo, L., Vrålstad, T., & Pennanen, T. (2005). UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol.* **166**:1063-1068.

Koide, R. T., Sharda, J. N., Herr, J. R., & Malcolm, G. M. (2008). Ectomycorrhizal fungi and the biotrophy–saprotrophy continuum. *New Phytol.* **178**:230-233.

Kuss, P., Raidl, S., & Beenken, L. (2004). Cortinarius huronensis Ammirati & Smith var. huronensis+ Pinus rotundata Link. *Descriptions of Ectomycorrhizae* **7**:21-27.

Lacourt, I., Girlanda, M., Perotto, S., Del Pero, M., Zuccon, D., & Luppi, A. M. (2001). Nuclear ribosomal sequence analysis of Oidiodendron: towards a redefinition of ecologically relevant species. *New Phytol.* **149**:565-576.

Landell, M. F., Brandão, L. R., Barbosa, A. C., Ramos, J. P., Safar, S. V., Gomes, F. C., Sousa, F. M. P., Morais, P. B., Leonardo, B., Leoncini, O., Ribeiro, J. R., Fungisin, B., Takashima, M., Nakase, T., Lee, C-F., Vainstein, M. H., Fell, J. W., Scorzetti, M., Vishniac, H. S., Rosa, C. A., & Valente, P. (2014). Hannaella pagnoccae sp. nov., a tremellaceous yeast species isolated from plants and soil. *International Journal of Systematic and Evolutionary Microbiology* **64**:1970-1977.

Leake, J. R., & Read, D. J. (1990). The effects of phenolic compounds on nitrogen mobilisation by ericoid mycorrhizal systems. *Agriculture, Ecosystems & Environment* **29**:225-236

Leake, J. R., & Read, D. J. (1991). Experiments with ericoid mycorrhiza. *Methods in Microbiology* **23**:435-459.

Lewis, D. H. (1973). Concepts in fungal nutrition and the origin of biotrophy. *Biological Reviews* **48**:261-277.

Lindahl, B. D., Ihrmark, K., Boberg, J., Trumbore, S. E., Högberg, P., Stenlid, J., & Finlay, R. D. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.* **173**:611-620.

Lindahl, B. D., & Tunlid, A. (2015). Ectomycorrhizal fungi–potential organic matter decomposers, yet not saprotrophs. *New Phytol.* **205**:1443-1447.

Magyar, L., Beenken, L., & Jakucs, E. (1999). Inocybe heimii Bon+Fumana procumbens (Dun.) Gr. Godr. *Descriptions of Ectomycorrhizae* **4**:61-65.

Mahmood, S., Finlay, R. D., & Erland, S. (1999). Effects of repeated harvesting of forest residues on the ectomycorrhizal community in a Swedish spruce forest. *New Phytol*. **142**:577-585.

Majdi, H., Pregitzer, K., Moren, A. S., Nylund, J. E., & Ågren, G. I. (2005). Measuring fine root turnover in forest ecosystems. *Plant and Soil* 276:1-8.

Marschner, H., & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* **159**:89-102.

Massicotte, H. B., Melville, L. H., Peterson, R. L., & Molina, R. (2000). Comparative anatomy of ectomycorrhizas synthesized on Douglas fir by Rhizopogon spp. and the hypogeous relative Truncocolumella citrina. *New phytol.* **147**:389-400.

Matheny, P. B., Aime, M. C., Bougher, N. L., Buyck, B., Desjardin, D. E., Horak, E., Kropp, B. R., Lodge, D. J., Soytong, K., Trappe, J. M. and Hibbett, D. S. (2009). Out of the Palaeotropics? Historical biogeography and diversification of the cosmopolitan ectomycorrhizal mushroom family Inocybaceae. *Journal of Biogeography* **36**:577–592.

Mayerhofer, M. S., Kernaghan, G., & Harper, K. A. (2013a). The effects of fungal root endophytes on plant growth: a meta-analysis. *Mycorrhiza* **23**:119-128.

Mayerhofer, M., Fraser, E., & Kernaghan, G. (2013b). Acid protease production by fungal root endophytes. *Phytopathology* **103**:92-92.

Melin, E. (1922). On the mycorrhizas of Pinus sylvestris L. and Picea abies Karst. A preliminary note. *Journal of Ecology* **9**:254-257.

Menkis, A., Vasiliauskas, R., Taylor, A. F., Stenlid, J., & Finlay, R. (2005). Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. *Mycorrhiza* **16**:33-41.

Miller, S. L., & Miller Jr, O. K. (1984). Synthesis of Elaphomyces muricatus+ Pinus sylvestris ectomycorrhizae. *Canadian Journal of Botany* **62**:2363-2369.

Mleczko, P. (2004). Mycorrhizal and saprobic macrofungi of two zinc wastes in southern Poland. *Acta Biol. Cracov. Ser. Bot* **46**:25-38.

Molina, R., Massicotte, H., & Trappe, J. M. (1992). Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. *Mycorrhizal functioning: an integrative plant-fungal process*. Springer-Verlag: Berlin-Heidelberg.

Nadelhoffer, K. J., & Raich, J. W. (1992). Fine root production estimates and belowground carbon allocation in forest ecosystems. *Ecology* **73**:1139-1147.

Nilsson, R. H., Larsson, K. H., Larsson, E., & Kõljalg, U. (2006). Fruiting body-guided molecular identification of root-tip mantle mycelia provides strong indications of

ectomycorrhizal associations in two species of Sistotrema (Basidiomycota). *Mycological Research* **110**:1426-1432.

Norby, R. J., & Jackson, R. B. (2000). Root dynamics and global change: seeking an ecosystem perspective. *New Phytol*. **147**:3-12.

Nuytinck, J., Verbeken, A., Rinaldi, A. C., Leonardi, M., Pacioni, G., & Comandini, O. (2004). Characterization of Lactarius tesquorum ectomycorrhizae on Cistus sp. and molecular phylogeny of related European Lactarius taxa. *Mycologia* **96**:272-282.

O'Brien, H. E., Parrent, J. L., Jackson, J. A., Moncalvo, J. M., & Vilgalys, R. (2005). Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**:5544-5550.

O'Dell, T. E., Massicotte, H. B., & Trappe, J. M. (1993). Root colonization of *Lupinus latifolius* Agardh. and *Pinus contorta* Dougl. by *Phialocephala fortinii* Wang & Wilcox. *New Phytol.* **124**:93-100.

Oksanen, J., Kindt, R. & O'Hara, R.B. (2005). vegan: Community Ecology Package version 1.6-9.

Oliver, R. P., & Ipcho, S. V. (2004). *Arabidopsis* pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. *Molecular Plant Pathology* **5**:347-352.

Ohenoja, E., Wang, Z., Townsend, J. P., Mitchel, D., & Voitk, A. (2010). Northern species of earth tongue genus *Thuemenidium* revisited, considering morphology, ecology and molecular phylogeny. *Mycologia* **102**:1089-1095.

Öpik, M., Moora, M., Liira, J., & Zobel, M. (2006). Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology* **94**:778-790.

Osono, T., & Takeda, H. (2006). Fungal decomposition of *Abies* needle and *Betula* leaf litter. *Mycologia* **98**:172-179.

Ottosson, E., Kubartová, A., Edman, M., Jönsson, M., Lindhe, A., Stenlid, J., & Dahlberg, A. (2015). Diverse ecological roles within fungal communities in decomposing logs of *Picea abies*. *FEMS Microbiology Ecology* **91**:1-12.

Perfect, S. E., & Green, J. R. (2001). Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. *Molecular Plant Pathology* 2:101-108.

Peterson, R. L., Massicotte, H. B., & Melville, L. H. (2004). *Mycorrhizas: anatomy and cell biology.* CABI.

Peyronel, B. (1922). Richerche sulle micorize e sulla microflora ridcola normale dell plante. *Bolletin della Stazione Patologia Vegetale* **3**:121-123.

Pregitzer, K. S., Zak, D. R., Curtis, P. S., Kubiske, M. E., Teeri, J. A., & Vogel, C. S. (1995). Atmospheric CO2, soil nitrogen and turnover of fine roots. *New Phytol.* **129**:579-585.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* **321**:341-361.

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.

Rice, A. V., & Currah, R. S. (2006). *Oidiodendron maius*: Saprobe in *Sphagnum* peat, mutualist in ericaceous roots? In: *Microbial Root Endophytes*. Springer-Verlag: Berlin-Heidelberg.

Rineau, F., Roth, D., Shah, F., Smits, M., Johansson, T., Canbäck, B., Olsen, P. B., Persson, P., Grell, M. N., Lindquist, E. Grigoriev, I. V., Lange, L., & Tunlid, A. (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.

Ryan, M. G., Hubbard, R. M., Pongracic, S., Raison, R. J., & McMurtrie, R. E. (1996). Foliage, fine-root, woody-tissue and stand respiration in Pinus radiata in relation to nitrogen status. *Tree physiology* **16**:333-343.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* **109**:6241-6246.

Schulz, B., & Boyle, C. (2005). The endophytic continuum. *Mycological Research* **109**:661-686.

Schulz, B., & Boyle, C. (2006). *What are endophytes?* Springer: Berlin-Heidelberg

Schützendübel, A., & Polle, A. (2002). Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany* **53**:1351-1365.

Siu R. G. H. (1951). *Microbial decomposition of cellulose*. Reinhold Publ. Corp.: New York.

Smith, S. E., & Read, D. J. (1997). *Mycorrhizal symbiosis* 2nd edn. Academic Press: Cambridge.

Trappe, J. M. (1969) Mycorrhiza-forming Ascomycetes. *Proceedings of the First North American Conference on Mycorrhizae*. Department of Agriculture, Forest Service: USA.

Taylor, A. F. S., & Alexander, I. J. (1989). Ectomycorrhizal synthesis with an isolate of *Russula aeruginea*. *Mycological Research* **92**:103-107.

Taylor, A. F. S., & Alexander, I. J. (1991). Ectomycorrhizal synthesis with *Tylospora fibrillosa*, a member of the Corticiaceae. *Mycological Research* **95**:381-384.

Tedersoo, L., Kõljalg, U., Hallenberg, N., & Larsson, K. H. (2003). Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytol.* **159**:153-165.

Tedersoo, L., Pärtel, K., Jairus, T., Gates, G., Põldmaa, K., & Tamm, H. (2009). Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales. *Environmental Microbiology* **11**:3166-3178.

Tedersoo, L., & Smith, M. E. (2013). Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews* **27**:83-99.

Tierney, G. L., & Fahey, T. J. (2002). Fine root turnover in a northern Deciduous forest: a direct comparison of the radiocarbon and minirhizotron methods. *Canadian Journal of Forest Research* **32**:1692-1697.

Trappe, J. M. (1962). Fungus associates of ectotrophic mycorrhizae. *The Botanical Review* **28**:538-606.

Twieg, B. D., Durall, D. M., & Simard, S. W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytol.* **176**:437-447.

Upson, R., Read, D. J., & Newsham, K. K. (2009). Nitrogen form influences the response of *Deschampsia antarctica* to dark septate root endophytes. *Mycorrhiza* **20**:1-11.

Vogel-Mikuš, K., Pongrac, P., Kump, P., Nečemer, M., & Regvar, M. (2006). Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *Environmental Pollution* **139**:362-371.

Vogt, K. A., Grier, C. C., Meier, C. E., & Edmonds, R. L. (1982). Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. *Ecology* **63**:370-380.

Vogt, K. A., Vogt, D. J., Palmiotto, P. A., Boon, P., O'Hara, J., Asbjornsen, H. (1995). Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant and Soil* **187**:159-219.

Vrålstad, T., Myhre, E., & Schumacher, T. (2002). Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the Helotiales in burnt and metal polluted habitats. *New Phytol.* **155**:131-148.

Wagg, C., Pautler, M., Massicotte, H. B., & Peterson, R. L. (2008). The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae. *Mycorrhiza* **18**:103-110.

Wang, C. J. K., & Wilcox, H. E. (1985). New species of ectendomycorrhizal and pseudomycorrhizal fungi: *Phialophora finlandia*, *Chloridium paucisporum*, and *Phialocephala fortinii*. *Mycologia* **77**:951-958.

Wilcox, H. E., Ganmore-Neumann, R., & Wang, C. J. K. (1974). Characteristics of two fungi producing ectendomycorrhizae in *Pinus resinosa*. *Canadian Journal of Botany* **52**:2279-2282.

Whipps, J. M. (2004). Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany* **82**:1198-1227.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfland, J. J. Sninsky, & T. J. White (Eds.). *PCR protocols: A guide to methods and applications* (pp. 315–322). Academic Press: San Diego.

Zhang, Y. H., & Zhuang, W. Y. (2004). Phylogenetic relationships of some members in the genus *Hymenoscyphus* (Ascomycetes, Helotiales). *Nova Hedwigia* **78**:475-484.

CHAPTER 4

Research Synthesis and Concluding Remarks

Although the fine root fungal communities examined in this study contained a combination of functional groups, ectomycorrhizal fungi were dominant. The five most abundant ectomycorrhizal genera according to the molecular data were *Piloderma, Cortinarius, Cenococcum, Russula,* and *Clavulina.* According to morphological observations, host senescence leads to communities dominated by the highly melanised mantles of *C. geophilum* and hyaline species decrease rapidly, with *Piloderma* and Russulaceae showing intermediate rates of disappearance. These discrepancies may have a few explanations. The dominance of *C. geophilum* mantles in the morphological data is likely due to its characteristic high melanin content and its extreme recalcitrance. As *Piloderma* and *Russula* ectomycorrhiza are difficult to identify compared to those formed by *C. geophilum,* there was surely some degree of identification bias toward *C. geophilum.* The increased abundance of *Piloderma* and *Russula* in the molecular data may be from tissues produced for saprotrophy; many of these structures could have been within the fine roots and would not have been visible during the morphological observations. Regarding similarities, *Cortinarius* was found to decrease in abundance after host senescence in both the molecular and morphological (hyaline category) data.

Endophytes showed an increase in abundance upon host death which began to subside at the last time interval (Figure 1). The most abundant endophyte was Helotiaceae sp. which showed up almost entirely in *A. balsamea.* In contrast, *P. rubens*

had approximately half (n = 23) the abundance of endophytes as *A. balsamea* (n = 39) and its endophytic community largely consisted of *Meliniomyces* and *Rhizoscyphus*. It may be that the increase in endophytic abundance is a result of increased availabilities of labile compounds upon fine root senescence. It could also be that endophytic fungi are increasing their production of exploratory structures used to abandon their senescent host to find a viable one. Given the variability of endophytes and the mixed endophytic community, a combination of both these behaviours is likely possible. Endophytes can be imagined as existing on a biotroph-saprotroph continuum with some more adapted to a semi-saprotrophic lifestyle than others as some have shown the capacity to decompose cellulose and hemicelluloses. As plant litter older than three years has been shown to have low levels of cellulose and hemicellulose (Osono & Takeda 1998), it may be that endophytes are accessing these resources. The abundance of Helotiaceae sp. may be because it has increased saprotrophic ability compared to other endophytes. More detailed studies centred on examining the endophytic community in fine roots are necessary to better understand this complex community of fungi. As morphological identification of endophytes is often difficult, future studies will likely need to be molecularly based.

Saprotrophs were not well represented in this study and appeared in low abundances, mostly at mid to late time intervals. The exception to this was *Galerina* which was both the most abundant and the earliest saprotroph detected in treatment seedlings. It is possible that some species of *Galerina* may have endophytic stages in coniferous fine roots as species of this genus have been detected in healthy bryophyte

tissues (Heimdal 2012; Gulden 2008) and the closely related genus *Mycena* has been found to contain endophytic species (Kernaghan & Patriquin 2011). Furthermore, *Galerina* was found almost entirely in *A. balsamea* which had a higher endophytic abundance compared to *P. rubens*. If *Galerina* were classified as an endophyte in this study, the saprotrophs would be almost negligible as OTUs of *Galerina* comprised nearly half of this category (Figure 2). Ecologically, the advantage gained by saprotrophic endophytes is spatiotemporal; these fungi would have access to senescent tissues before externally living saprotrophs. This could potentially lead to faster decomposition of fine roots harbouring saprotrophic endophytes compared to those fine roots lacking them.

Longer studies focusing on the fungal dimension of fine root decomposition are needed. As fine root turnover deposits large amounts of carbon into forest soils, better understanding of the dynamics of fine root decomposition is crucial to more accurate carbon modelling. This is especially important in northern forests as decomposition of SOM is characteristically slow (Berg 2000; Berg et al. 2000), as our edaphic measurements show (Chapter 2). Future studies should concentrate on measuring the labile (sugars, proteins, cellulose, hemicelluloses) and recalcitrant (lignin, suberin, waxes) compounds of fine roots and molecular characterisation of the fungal communities. This would allow for more confident categorization of endophytes and traditional saprophytes into functional niches of the decomposition process.

In summary, endophytes were found to be less common than ectomycorrhizae, but they were far more common than saprotrophs. This may indicate that endophytes play a role in early fine root decomposition, before saprotrophic fungi. The dominance of

some types of ectomycorrhizal fungi likely also play a role in decomposition, whether this be by decreasing decomposition through the presence of recalcitrant compounds or by increasing it by their own saprotrophic abilities. The differences between fine root loss in *A. balsamea* and *P. rubens* has shown that differences in ecologically dominant tree species may have a significant effect on the amount or rate that fine root litter enters forest soils. Although limited in sample size compared to next generation sequencing, the traditional cloning methods used in this study have provided important data regarding the fungal communities of the early stages of decomposition in *A. balsamea* and *P. rubens* fine roots. Some ectomycorrhizal fungi appear to tolerate host death better than others and endophytic fungi may play roles in decomposition. Whether these roles are only until saprotrophic fungi dominate the fine root litter is unknown.

As climate change is undoubtedly the most important scientific issue on the horizon, it is fundamentally important that the organisms involved in fine root decomposition are well understood. A better understanding of how fungi affect fine root decomposition of northern forests may increase carbon model accuracy by allowing a more accurate calculation of fine root decomposition rate. The baseline data produced by this study are an important start in characterising how root associated fungal communities are affected by fine root decomposition and how these communities may impact fine root decomposition. The results of this research can act as a springboard for future research into the roles that endophytic and ectomycorrhizal fungi play in fine root decomposition.

References

Berg, B. (2000). Litter decomposition and organic matter turnover in northern forest soils. *Forest ecology and Management* **133**:13-22.

Berg, B., Meentemeyer, V., & Johansson, M.-B. (2000). Litter decomposition in a transect of Norway spruce forests; substrate quality and climate control. *Can. J. For. Res.* **30**:1136-1147.

Gulden, G. (2008). *Galerina earle*. In H. Knudsen & J Vesterholt (Eds.), *Funga Nordica: Agaricoid, Boletoid, and Cyphelloid Genera* (pp. 785-804). Nordsvamp: Copenhagen.

Heimdal, R. S. (2012). *Using 454-sequencing for exploring diversity, host specificity and tissue specificity of the fungal genus* Galerina *associated with four boreal mosses* (Masters dissertation). Retrieved from University of Oslo (duo.uio.no).

Kernaghan, G., & Patriquin, G. (2011). Host associations between fungal root endophytes and boreal trees. *Microbial Ecology* **62**:460-473.

Keuskamp, J. A., Dingemans, B. J. J., Lehtinen, T., Sarneel, J. M., & Hefting, M. M. (2013). Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. *Methods in Ecology and Evolution* **4**:1070-1075.

Osono, T., & Takeda, H. (2006). Fungal decomposition of *Abies* needle and *Betula* leaf litter. *Mycologia* **98**:172-179.

APPENDIX 1

Supplemental Materials for Chapter 2

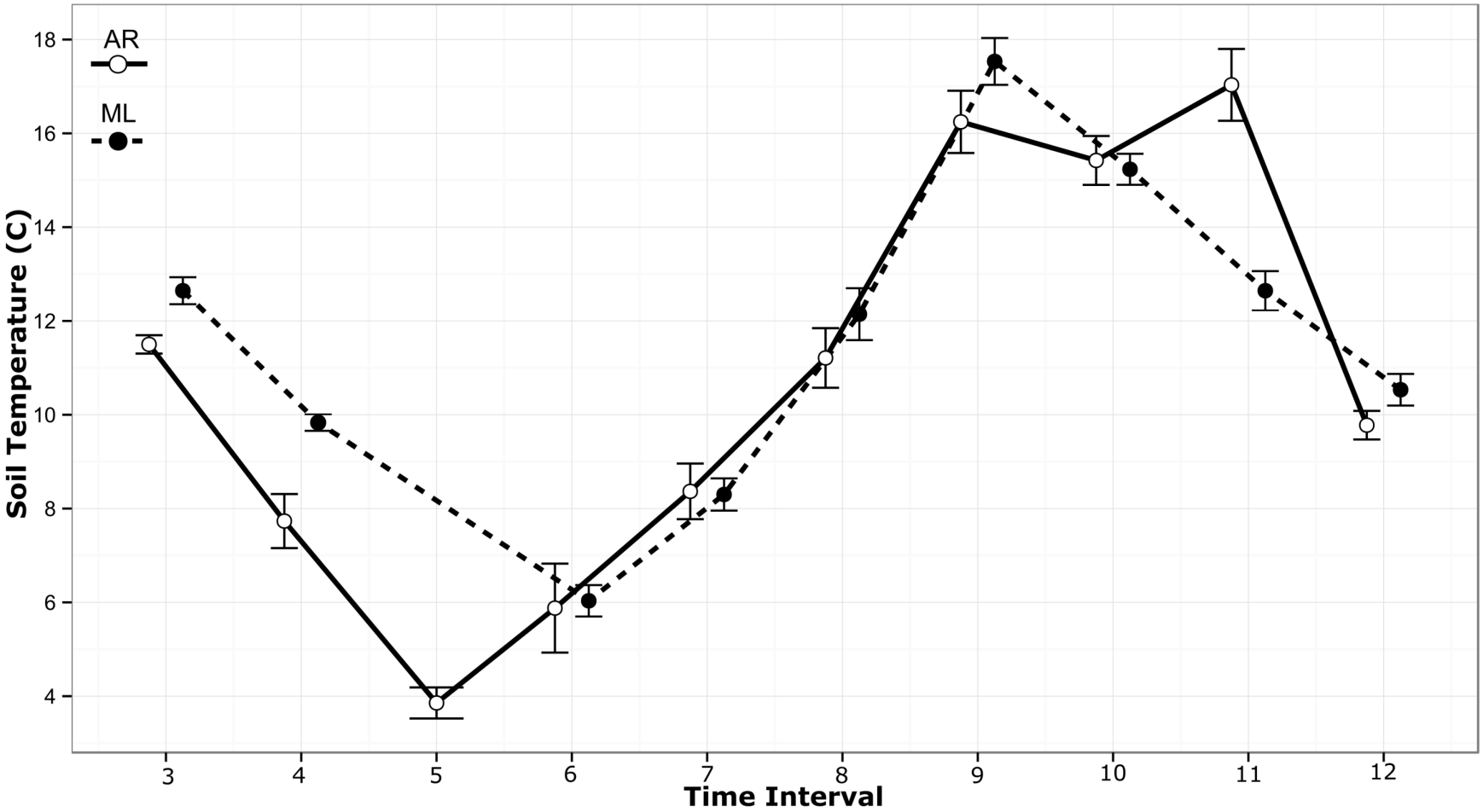

Figure 1. Mean soil temperature for Annapolis Road (AR) and McGowan Lake (ML). Error bars represent 95% confidence interval.

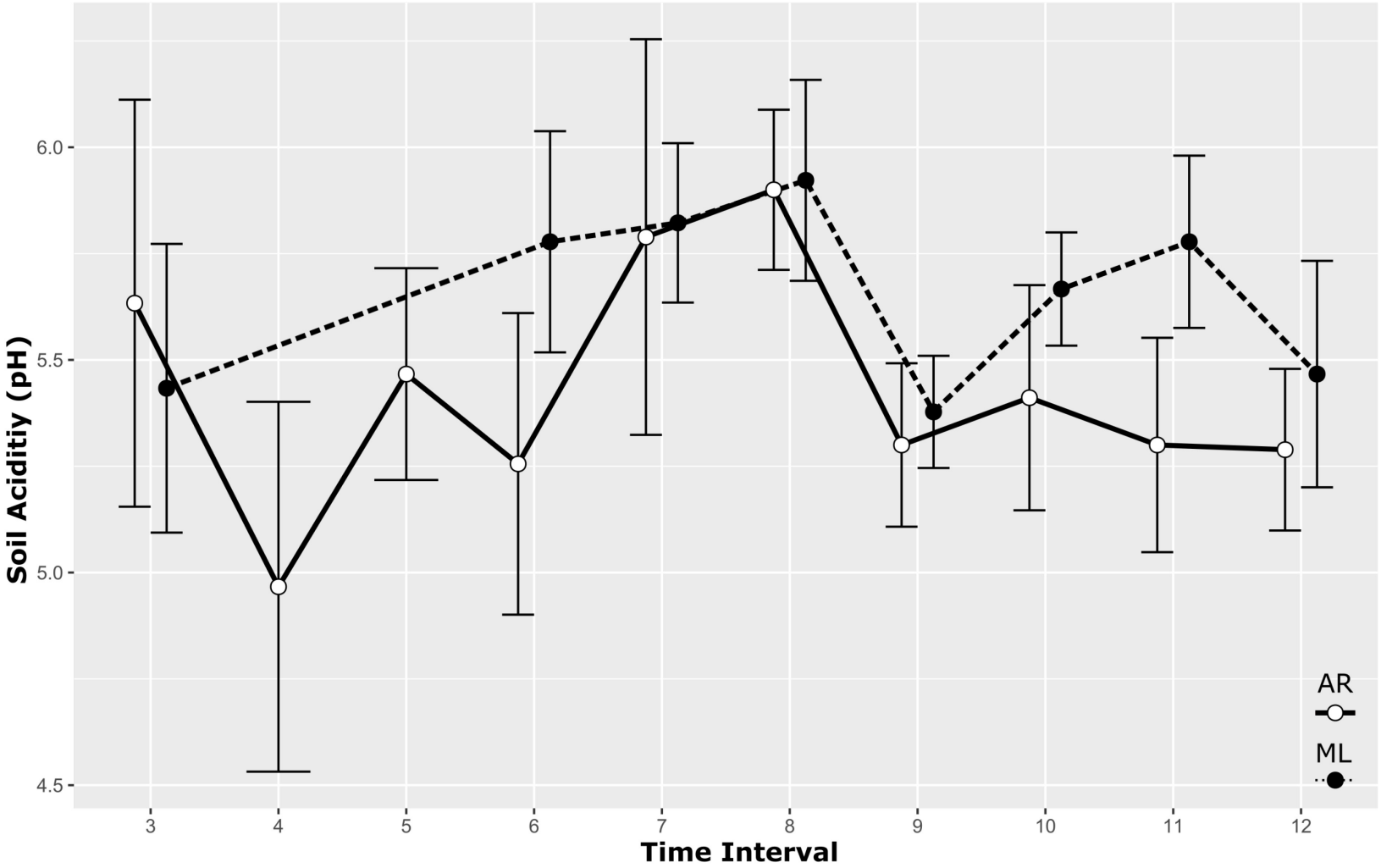

Figure 2. Mean soil acidity for Annapolis Road (AR) and McGowan Lake (ML): error-bars represent 95% confidence intervals.

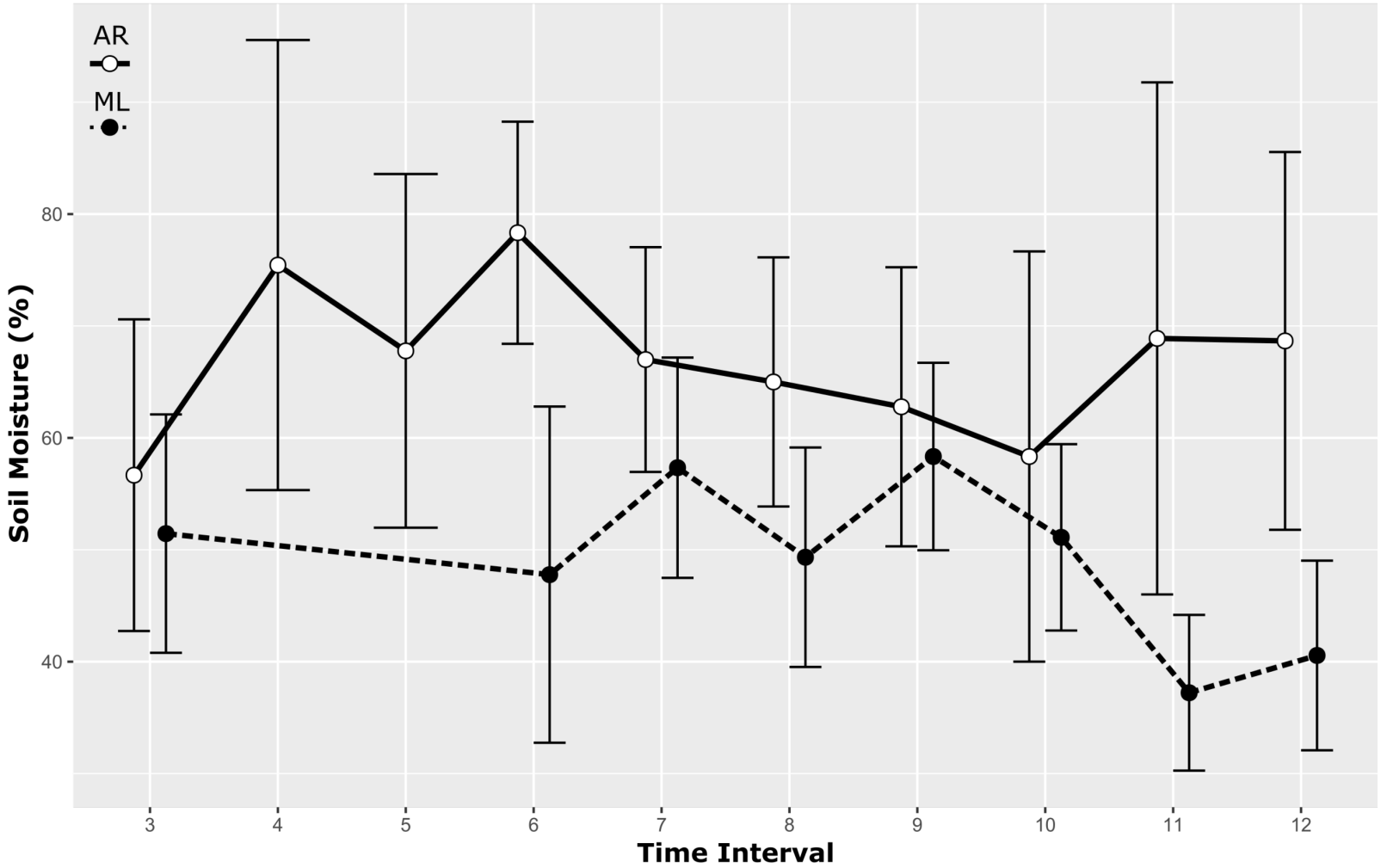

Figure 3. Mean soil moisture for Annapolis Road (AR) and McGowan Lake (ML): error-bars represent 95% confidence intervals.

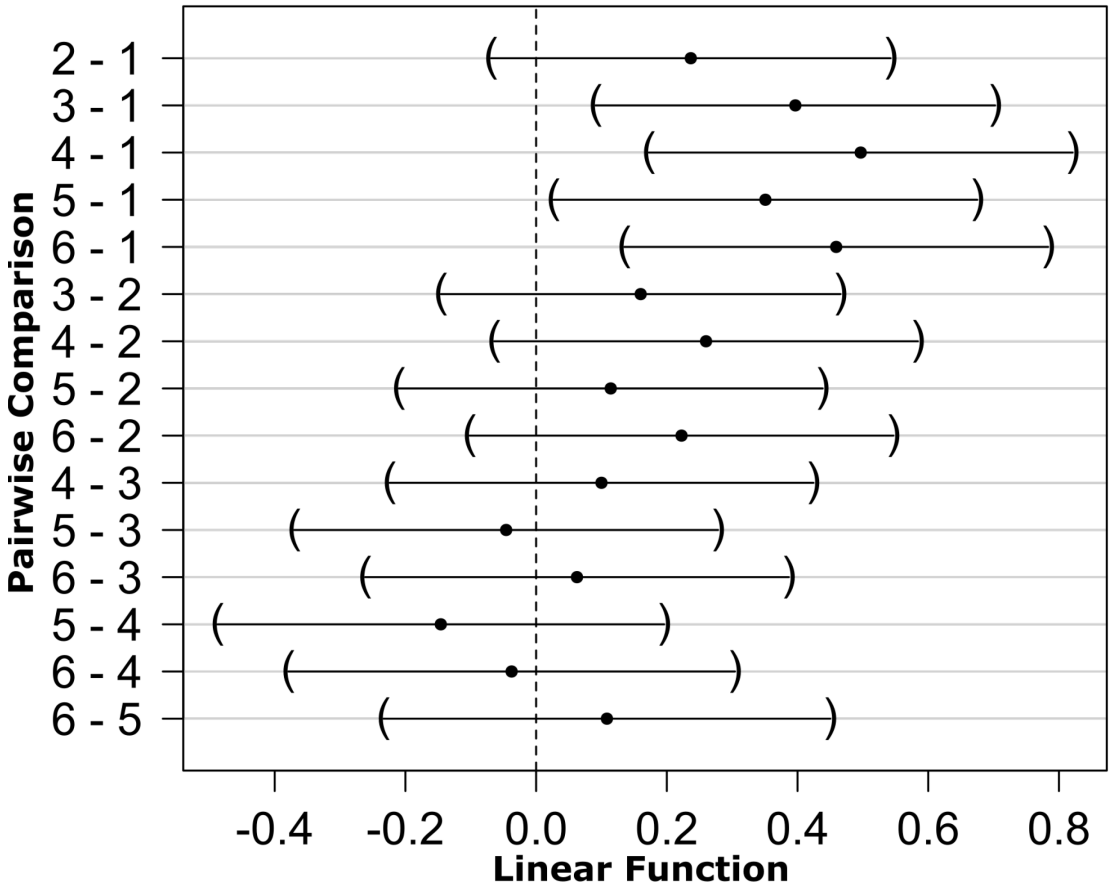

Figure 4. 95% family wise confidence levels for pH ~ plot at Annapolis Road (1, 2, 3) and McGowan Lake (4, 5, 6).

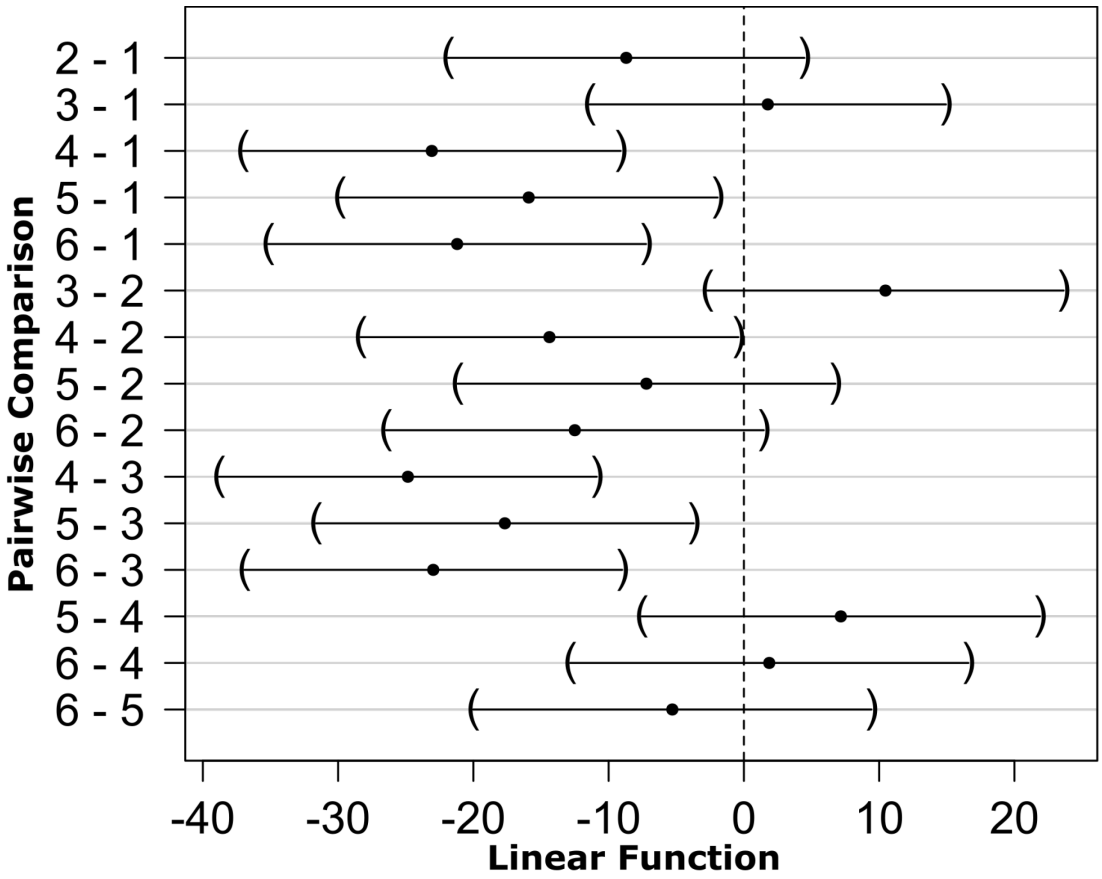

Figure 5. 95% family wise confidence levels for soil moisture | plot at Annapolis Road (1, 2, 3) and McGowan Lake (4, 5, 6).

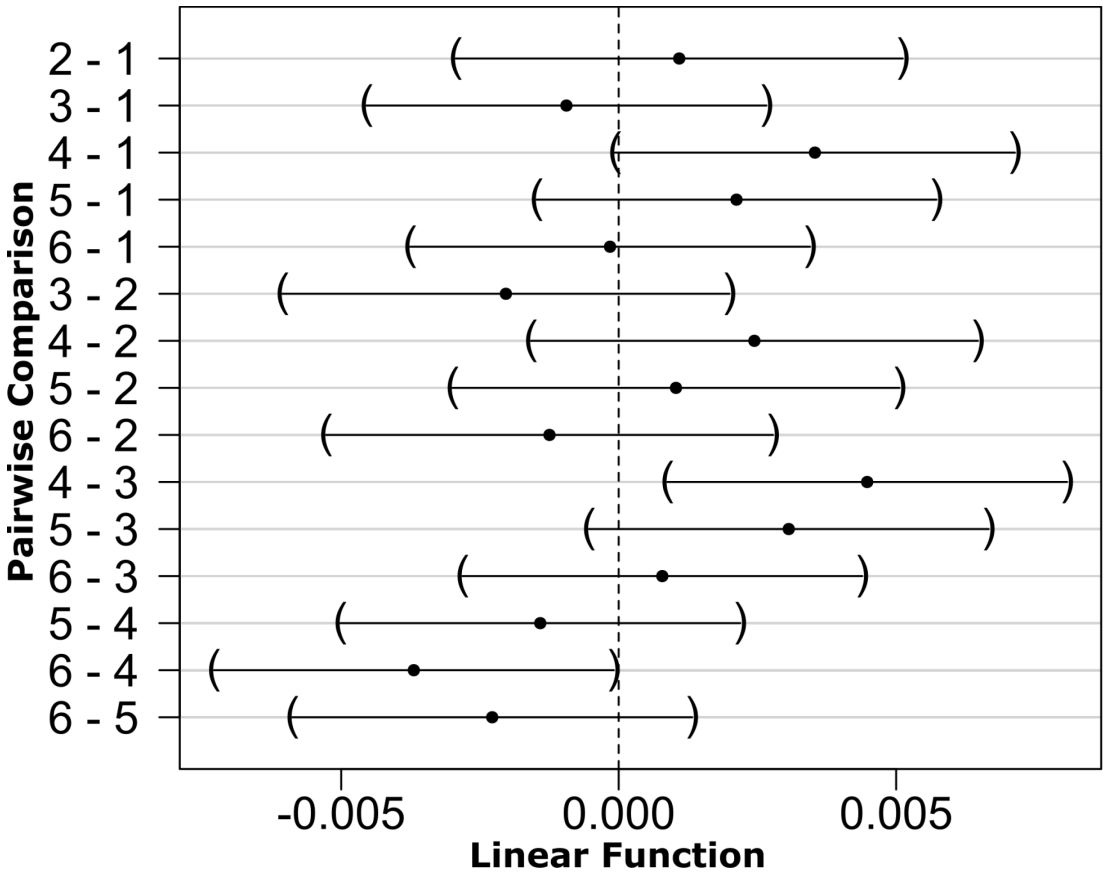

Figure 6. 95% family wise confidence levels for TBI ~ plot at Annapolis Road (1, 2, 3) and McGowan Lake (4, 5, 6)

.

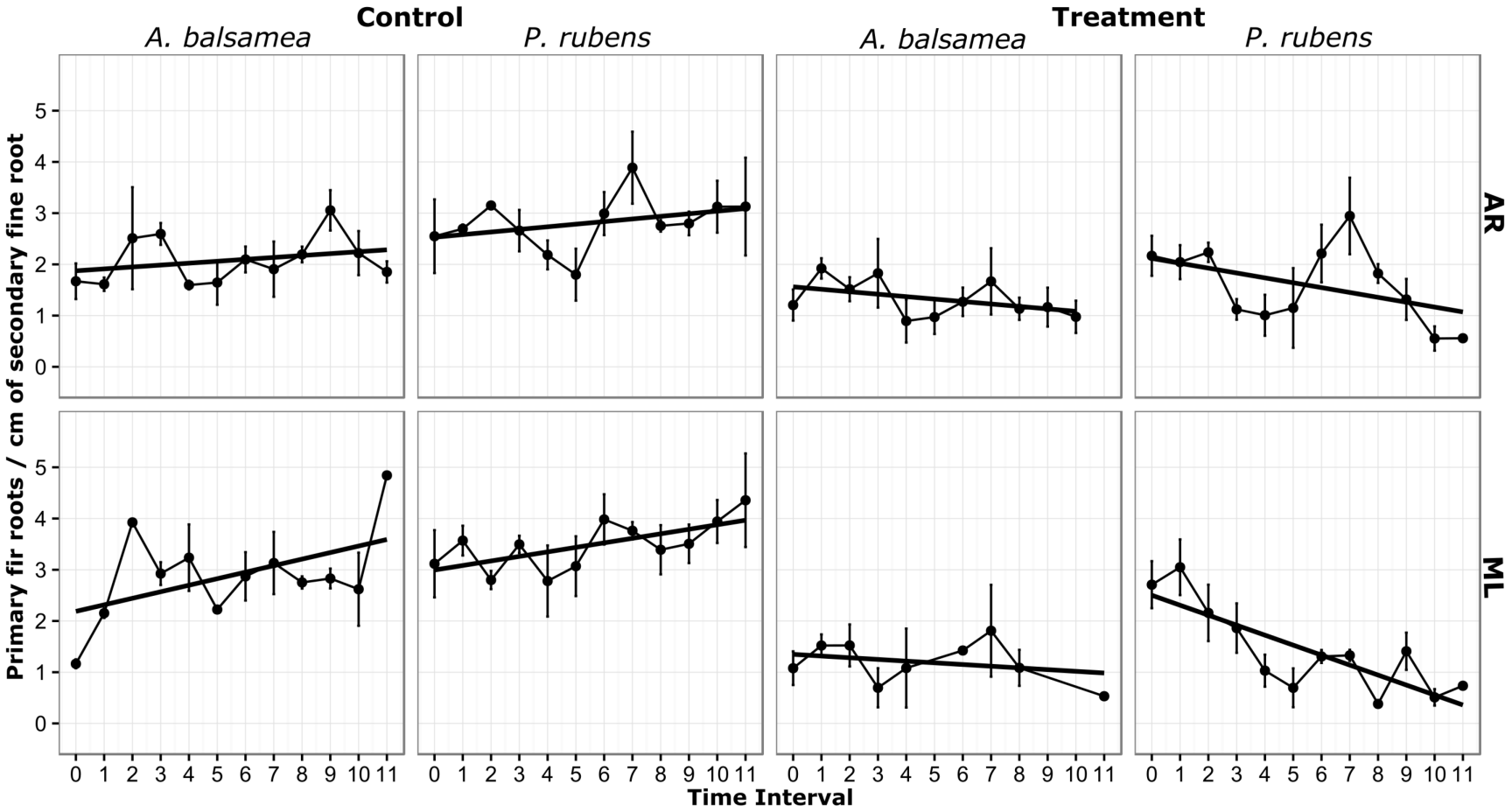

Figure 7. Primary fine root loss in control and treatment seedlings of *A. balsamea* and *P. rubens* at Annapolis Road (AR) and McGowan Lake (ML): error-bars = SE.

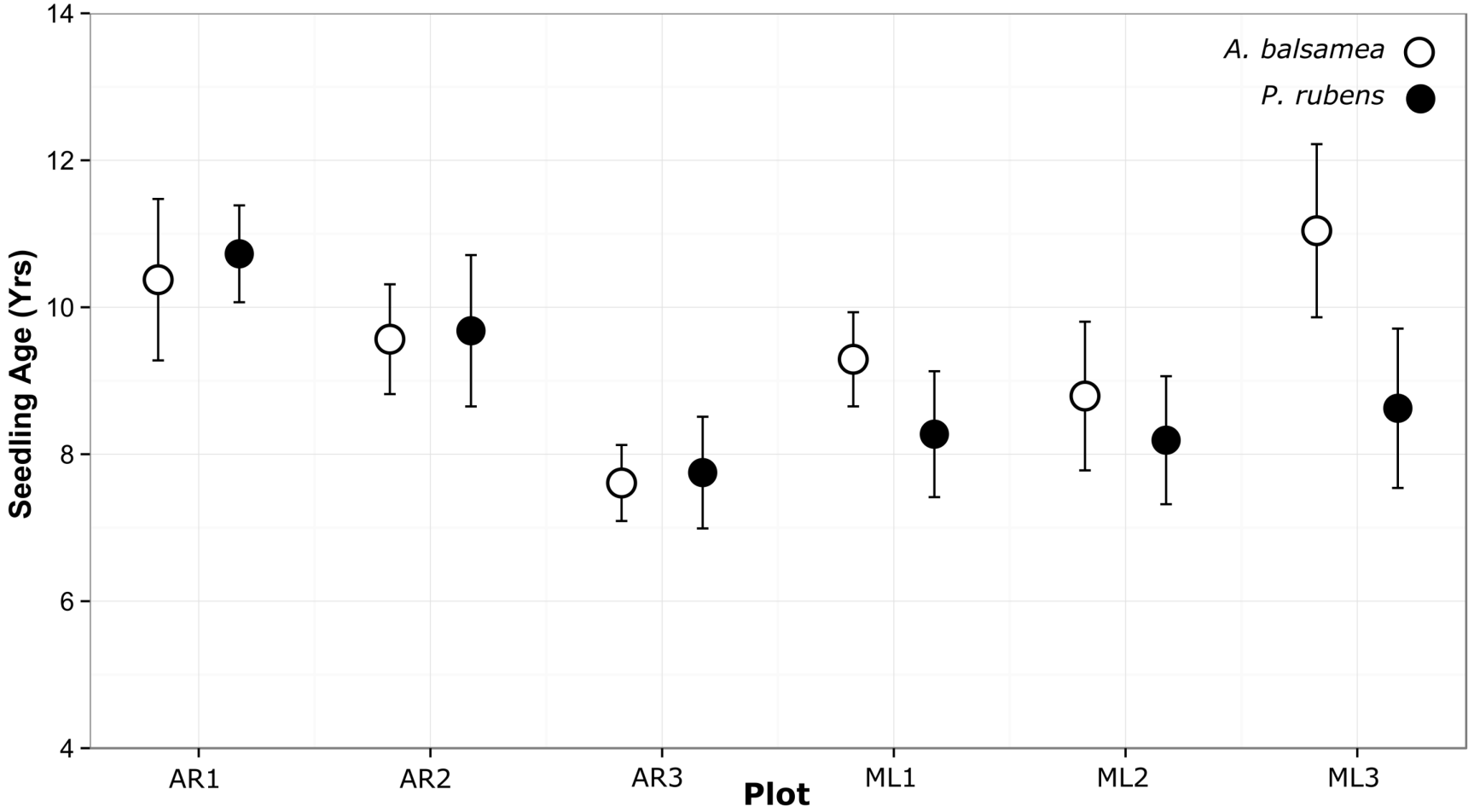

Figure 8. Seedling age for *A. balsamea* and *P. rubens* at each site: error-bars represent 95% confidence intervals.

.

Table 1. Edaphic measurements for Annapolis Road Plot 1.

Time Interval	Soil pH		Soil Temp. (°C)		Soil Moisture (%)	
	Rep.	Avg.	Rep.	Avg.	Rep.	Avg.
0	-	-	-	-	-	-
	-		-		-	
	-		-		-	
1	-	-	-	-	-	-
	-		-		-	
	-		-		-	
2	-	-	-	-	-	-
	-		-		-	
	-		-		-	
3	5.2	5.2	11.8	11.7	60	70
	4.6		11.7		80	
	5.8		11.5		60	
4	3.9	4.5	8.4	8.2	90	80
	5.0		7.5		60	
	4.5		8.7		85	
5	5.0	5.2	4.0	3.8	50	60
	5.1		3.3		70	
	5.6		4.1		60	
6	5.5	5.2	5.7	5.4	70	75
	5.1		5.6		95	
	5.1		5.0		60	
7	4.2	5.3	8.7	8.1	70	80
	5.9		6.9		80	
	5.9		8.6		90	
8	6.0	5.8	11.6	11.3	70	75
	5.8		10.7		60	
	5.5		11.6		90	
9	5.6	5.4	15.5	15.4	65	50
	5.5		15.0		60	
	5.2		16.3		40	
10	5.2	5.3	14.9	14.7	55	70
	5.2		14.3		100	
	5.4		15.0		60	
11	5.2	5.1	17.8	17.1	90	65
	5.0		16.7		80	
	5.2		16.8		25	
12	5.2	5.1	10.1	10.0	40	65
	5.0		10.0		100	
	5.2		9.9		60	

Table 2. Edaphic measurements for Annapolis Road Plot 2.

Time Interval	Soil pH		Soil Temp. (°C)		Soil Moisture (%)	
	Rep.	Avg.	Rep.	Avg.	Rep.	Avg.
0	-	-	-	-	-	-
	-		-		-	
	-		-		-	
1	-	-	-	-	-	-
	-		-		-	
	-		-		-	
2	-	-	-	-	-	-
	-		-		-	
	-		-		-	
3	5.8	5.9	11.3	11.6	30	40
	5.2		11.8		60	
	6.7		11.6		25	
4	5.4	5.0	8.5	7.8	35	55
	5.0		7.5		40	
	4.5		7.4		100	
5	5.8	5.5	4.0	4.3	90	70
	5.3		4.5		80	
	5.4		4.3		40	
6	5.6	5.2	5.5	7.0	70	80
	4.3		8.4		95	
	5.8		7.0		70	
7	6.0	6.0	8.6	8.6	70	60
	6.0		8.6		50	
	5.9		8.7		60	
8	6.1	5.9	11.0	11.7	45	65
	5.5		11.1		70	
	6.0		13.0		75	
9	5.0	5.1	16.4	16.9	55	60
	5.0		17.6		60	
	5.3		16.8		70	
10	4.7	5.4	15.0	15.5	45	50
	5.8		15.4		55	
	5.7		16.1		50	
11	4.8	5.2	17.8	17.6	95	60
	5.1		17.3		40	
	5.8		17.8		45	
12	5.6	5.4	10.5	9.7	70	70
	5.0		9.3		90	
	5.6		9.3		45	

Table 3. Edaphic measurements for Annapolis Road Plot 3.

Time Interval	Soil pH		Soil Temp. (°C)		Soil Moisture (%)	
	Rep.	Avg.	Rep.	Avg.	Rep.	Avg.
0	-	-	-	-	-	-
	-		-		-	
	-		-		-	
1	-	-	-	-	-	-
	-		-		-	
	-		-		-	
2	-	-	-	-	-	-
	-		-		-	
	-		-		-	
3	6.0	5.8	11.5	11.2	60	65
	6.1		11.2		60	
	5.3		11.1		70	
4	5.6	5.5	8.0	7.2	70	90
	5.3		7.3		100	
	5.5		6.3		100	
5	5.3	5.7	3.5	3.5	80	75
	5.8		3.3		100	
	5.9		3.7		50	
6	5.0	5.3	4.3	5.2	90	80
	5.7		6.4		70	
	5.2		5.0		85	
7	5.9	6.1	7.4	8.4	65	60
	6.2		9.5		50	
	6.1		8.3		70	
8	6.0	6.1	10.8	10.6	50	60
	6.1		10.0		75	
	6.1		11.1		50	
9	5.7	5.4	17.2	16.2	55	70
	5.2		15.4		60	
	5.2		16.0		100	
10	5.4	5.6	15.9	16.0	90	55
	5.6		16.4		20	
	5.7		15.8		50	
11	5.7	5.5	18.0	16.4	45	80
	5.5		15.0		100	
	5.4		16.1		100	
12	5.6	5.3	9.6	9.6	60	70
	5.2		9.8		60	
	5.2		9.5		95	

Table 4. Edaphic measurements for McGowan Lake Plot 1.

Time Interval	Soil pH		Soil Temp. (°C)		Soil Moisture (%)	
	Rep.	Avg.	Rep.	Avg.	Rep.	Avg.
0	-	-	-	-	-	-
	-		-		-	
	-		-		-	
1	-	-	-	-	-	-
	-		-		-	
	-		-		-	
2	-	-	-	-	-	-
	-		-		-	
	-		-		-	
3	6.2	5.6	12.2	12.7	45	45
	5.6		13.0		45	
	5.0		12.8		50	
4	-	-	10.0	9.8	-	-
	-		9.5		-	
	-		9.8		-	
5	-	-	3.3	-	-	-
	-		3.5		-	
	-		3.4		-	
6	6.0	5.7	5.8	6.0	50	55
	5.5		6.3		65	
	5.5		5.8		50	
7	6.0	5.4	7.9	8.1	65	55
	5.6		8.1		60	
	6.1		8.4		40	
8	6.1	6.0	12.1	12.2	50	40
	6.0		11.9		40	
	5.8		12.6		30	
9	5.5	5.4	18.4	17.7	45	55
	5.2		17.2		60	
	5.5		17.4		60	
10	5.6	5.7	15.3	15.4	50	40
	5.6		15.4		50	
	5.8		15.4		30	
11	6.0	5.8	12.3	12.4	20	30
	5.3		12.4		40	
	6.0		12.5		30	
12	6.2	5.8	10.7	10.6	40	45
	5.8		10.9		40	
	5.3		10.2		50	

Table 5. Edaphic measurements for McGowan Lake Plot 2.

Time Interval	Soil pH		Soil Temp. (°C)		Soil Moisture (%)	
	Rep.	Avg.	Rep.	Avg.	Rep.	Avg.
0	-	-	-	-	-	-
	-		-		-	
	-		-		-	
1	-	-	-	-	-	-
	-		-		-	
	-		-		-	
2	-	-	-	-	-	-
	-		-		-	
	-		-		-	
3	5.4	5.4	12.9	12.4	85	60
	5.2		12.4		45	
	5.6		12		50	
4	-	-	9.7	9.9	-	-
	-		10.1		-	
	-		9.8		-	
5	-	-	3.6	-	-	-
	-		3.4		-	
	-		3.7		-	
6	5.4	5.5	6.5	6.4	85	60
	5.5		5.9		45	
	5.7		6.9		40	
7	5.8	5.6	8.6	8.8	80	65
	5.5		8.7		55	
	5.5		9		65	
8	5.5	6	13	12.7	75	60
	6.5		11.7		50	
	6		13.3		60	
9	5.2	5.3	16.8	17.5	60	60
	5.6		17.9		40	
	5.2		17.8		75	
10	5.4	5.7	14.5	14.8	55	50
	5.8		14.7		60	
	5.8		15.2		40	
11	5.9	5.7	12	12.8	50	40
	5.8		13.8		30	
	5.4		12.5		40	
12	5.3	5.3	9.7	10.3	25	30
	5.3		10.9		40	
	5.4		10.2		30	

Table 6. Edaphic measurements for McGowan Lake Plot 3.

Time Interval	Soil pH		Soil Temp. (°C)		Soil Moisture (%)	
	Rep.	Avg.	Rep.	Avg.	Rep.	Avg.
0	-	-	-	-	-	-
	-		-		-	
	-		-		-	
1	-	-	-	-	-	-
	-		-		-	
	-		-		-	
2	-	-	-	-	-	-
	-		-		-	
	-		-		-	
3	4.7	5.3	12.8	12.8	50	45
	5.4		13.1		55	
	5.8		12.6		35	
4	-	-	9.8	9.9	-	-
	-		10.2		-	
	-		9.6		-	
5	-	-	3.6	-	-	-
	-		3.8		-	
	-		3.8		-	
6	6.4	6.1	5.7	5.7	25	35
	6.0		5.6		50	
	6.0		5.8		20	
7	5.8	6.0	7.7	8.0	60	50
	6.1		7.8		40	
	6.0		8.5		50	
8	5.5	5.8	12.2	11.6	50	45
	5.9		11.3		50	
	6.0		11.2		40	
9	5.5	5.4	16.8	17.4	55	60
	5.2		17.0		60	
	5.5		18.5		70	
10	5.8	5.7	15.1	15.5	60	60
	5.4		15.6		65	
	5.8		15.9		50	
11	5.7	5.9	12.3	12.8	45	40
	5.9		13.1		40	
	6.0		12.9		40	
12	5.4	5.3	10.6	10.7	55	45
	5.5		10.5		30	
	5.0		11.1		55	

Table 7. Initial and final mass of oven dried green and rooibos teas (without bag) after approximately 90 days post burial.

Site	Plot	$Initial_g$(g)	$Final_g$(g)	$Loss_g$(g)	a_g	$Initial_r$(g)	$Final_r$(g)	$Loss_r$(g)	a_r	S	k
Annapolis Road	1	1.631	0.664	0.967	0.593	2.118	1.751	0.367	0.389	0.296	0.008
		1.650	0.676	0.974	0.590	2.141	1.748	0.393	0.387	0.299	0.008
		1.545	0.603	0.942	0.610	2.148	1.809	0.339	0.400	0.276	0.007
	2	1.591	0.701	0.890	0.559	2.144	1.724	0.42	0.367	0.336	0.009
		1.591	0.748	0.843	0.530	2.145	1.809	0.336	0.347	0.371	0.007
		-	-	-	-	-	-	-	-	-	-
	3	1.582	0.705	0.877	0.554	2.149	1.791	0.358	0.363	0.342	0.007
		1.644	0.634	1.010	0.614	2.142	1.845	0.297	0.403	0.270	0.006
		1.633	0.660	0.973	0.596	2.142	1.847	0.295	0.391	0.292	0.006
McGowan Lake	1	1.585	0.774	0.811	0.512	2.144	1.665	0.479	0.335	0.392	0.01
		1.622	0.808	0.814	0.502	2.141	1.777	0.364	0.329	0.404	0.007
		1.638	0.780	0.858	0.524	2.146	1.702	0.444	0.343	0.378	0.01
	2	1.615	0.716	0.899	0.557	2.144	1.686	0.458	0.365	0.339	0.01
		1.615	0.773	0.842	0.521	2.139	1.763	0.376	0.342	0.381	0.008
		1.595	0.794	0.801	0.502	2.138	1.771	0.367	0.329	0.404	0.007
	3	1.668	0.775	0.893	0.535	2.143	1.807	0.336	0.351	0.364	0.007
		1.584	0.754	0.830	0.524	2.142	1.778	0.364	0.344	0.378	0.007
		1.626	0.524	1.102	0.678	2.143	1.818	0.325	0.444	0.195	0.006

Table 8. Descriptions of plots.

Site	Plot	Canopy	Notes
Annapolis Road	1	~ 50% Deciduous ~ 50% Coniferous	Conifers mostly *A. balsamea* Deciduous mostly *Acer* sp. Granite stones (20 to 70 cm) covered ~ 25% of surface
	2	> 75% Coniferous	Conifers mostly *P. rubens,* some *A. balsamea* Deciduous mostly *Betula* spp.
	3	~ 100% Coniferous	Conifers almost entirely *A. balsamea* with some *P. rubens* Granite stones (30 to 60 cm) covered between 25 and 50% of surface
McGowan Lake	1	~ 70% Deciduous ~ 30% Coniferous	Conifers mostly *A. balsamea* Deciduous mostly *Acer* spp. with *Betula* sp. and *Fagus* sp. present
	2	> 75% Coniferous	Conifers equal between *A. balsamea* and *P. rubens*; lots of *Pinus* sp. Deciduous mainly *Betula* sp.
	3	~ 50% Deciduous ~ 50% Coniferous	Conifers mostly *A. balsamea* Deciduous mostly *Acer* with some *Quercus* sp.

APPENDIX 2

Supplemental Materials for Chapter 3

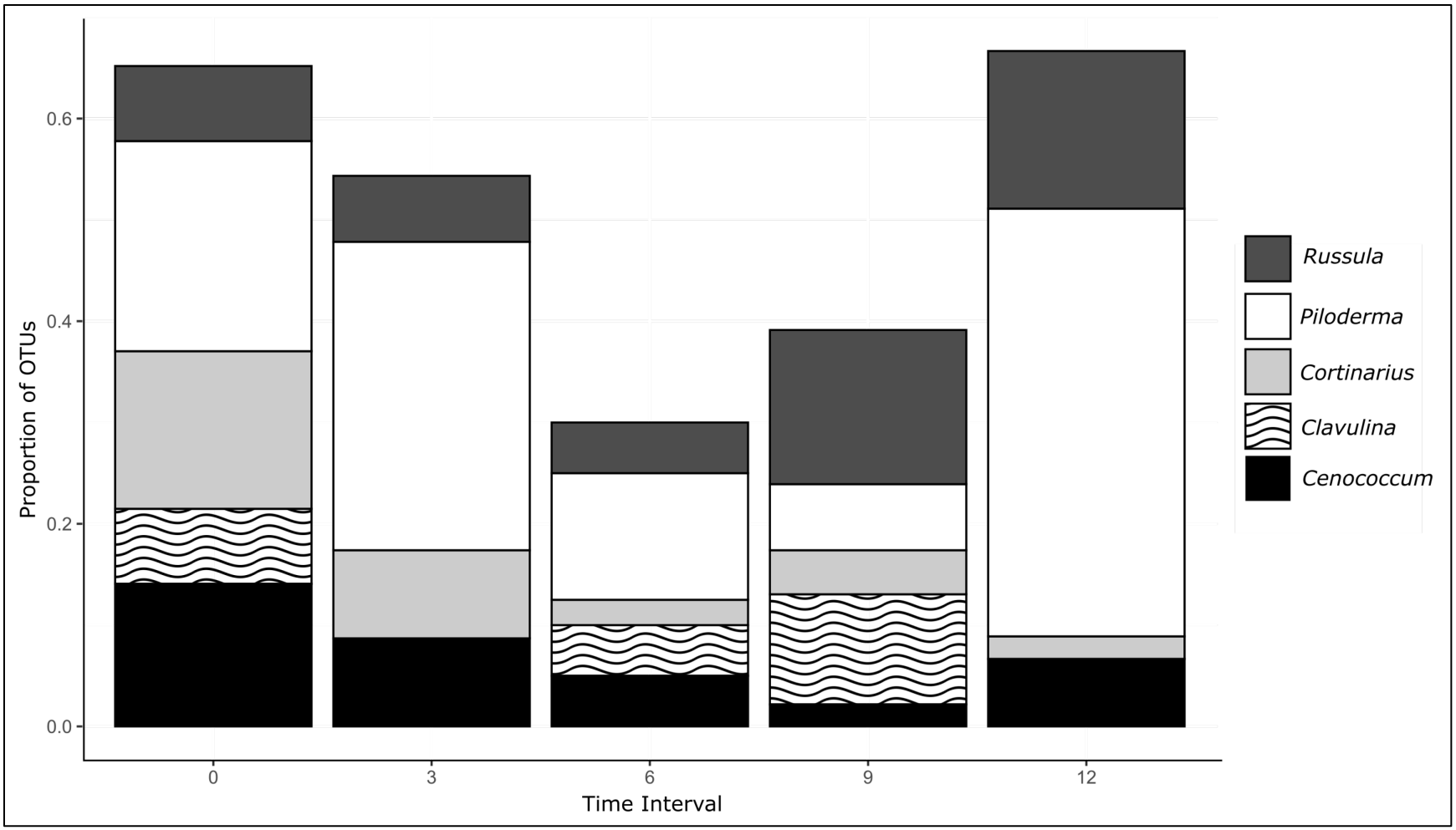

Figure 1. Proportion of the fungal community made up by the most common ectomycorrhizal genera in treatment seedlings.

Table 1. Collection dates and months post treatment for time intervals. Underlined dates denote time intervals used for metagenomic analysis, dates with asterisks were used for morphological data.

Collection date (DD-MM-YY)	Time interval	Months post treatment
21-06-13*	0	0
21-07-13	1	1
21-08-13*	2	2
21-09-13	3	3
21-10-13*	4	4
21-11-13	5	5
Overwintering period	N/A	6, 7, 8, 9
21-04-14	6	10
21-05-14*	7	11
21-06-14	8	12
21-07-14	9	13
21-08-14*	10	14
21-09-14	11	15
22-10-14	12	16

Table 2. OTUs assigned to sequences from primary fine roots of *A. balsamea* from Annapolis Road and McGowan Lake. Numbers in parenthesis indicate abundance of the OTU in each sample.

Site	Time Interval 3	Time Interval 6	Time Interval 9	Time Interval 12
AR	*Galerina* sp. (7) Helotiaceae sp. VI (2) *Meliniomyces variabilis* (2) *Russula fragilis* (1)	Helotiaceae sp. VI (4) *Piloderma sphaerosporum* (3) *Galerina* sp. (2) *Mycena* sp. (1) *Phialocephala* sp. (1) *Geoglossum* sp. (1)	Helotiaceae sp. VI (4) *Sistotrema* sp. (2) *Amanita flavoconia* (1) *Amanita* sp. (1) *Hyphodiscus* sp. (1) *Galerina* sp. (1)	*Piloderma* sp. (4) Helotiaceae sp. VI (3) *Galerina* sp. (2) *Cortinarius* sp. (1) *Hannaella* sp. (1)
ML	*Clavulina cinerea* (4) *Oidiodendron* sp. (3) *Cenococcum geophilum* (2) *Rhizoscyphus ericae* (1) *Hyphodiscus* sp. (1)	Helotiaceae sp. VI (6) *Trechispora* sp. (2) *Elaphomyces* sp. (1) *Piloderma sphaerosporum* (1) *Cenococcum geophilum* (1)	*Russula* sp. (4) *Lactarius deceptivus* (3) *Chloridium* sp. (2) *Lactarius vinaceorufescens* (1) Helotiaceae sp. VI (1) *Perenniporia* sp. (1)	*Piloderma* sp. (7) *Cenococcum geophilum* (2) *Russula fragilis* (1) *Russula* sp. (1) *Chloridium* sp. (1)

Table 3. OTUs assigned to sequences from primary fine roots of *P. rubens* from Annapolis Road and McGowan Lake at various time intervals. Numbers in parenthesis indicate abundance of the OTU in each sample.

Site	Time Interval 3	Time Interval 6	Time Interval 9	Time Interval 12
AR	*Piloderma fallax* (6) *Cenococcum geophilum* (2) *Russula silvestris* (2) *Meliniomyces variabilis* (1)	*Meliniomyces variabilis* (6) *Rhizoscyphus ericae* (1) Hyaloscyphaceae sp. (1) Helotiales sp. (1) *Cortinarius* sp. (1) *Cenococcum geophilum* (1)	*Piloderma fallax* (2) *Camarophyllopsis* sp. (2) *Cortinarius* sp. (2) *Trechispora* sp. (2) Hyaloscyphaceae sp. (1) *Russula silvestris* (1) *Cenococcum geophilum* (1) *Piloderma sphaerosporum* (1)	*Hebeloma velutipes* (6) *Russula peckii* (2) *Russula fragilis* (1) *Cenococcum geophilum* (1) *Meliniomyces* sp. (1)
ML	*Piloderma sphaerosporum* (7) *Meliniomyces variabilis* (3) *Rhizoscyphus ericae* (2)	*Trechispora* sp. (4) *Clavulina cinerea* (2) *Russula vesca* (2) *Ramariopsis* sp. (1) *Piloderma* sp. (1) Hyaloscyphaceae sp. (1)	*Clavulina cinerea* (5) *Cryptococcus terricola* (2) *Galerina* sp. (2) *Russula* sp. (1) Herpotrichellaceae sp. (1) *Mortierella* sp. (1)	*Piloderma* sp. (8) *Inocybe* sp. (1) Helotiaceae sp. V (1) Hyaloscyphaceae sp. (1) *Russula fragilis* (1)

Table 4. OTUs assigned to sequences from primary fine roots of *A. balsamea* and *P. rubens* from Annapolis Road and McGowan Lake at Time Interval 0 (Controls). Number in parenthesis indicates the abundance of the OTU in each sample.

Site	Control – *A. balsamea*	Control – *P. rubens*
AR	*Cenococcum geophilum* (11) *Clavulina cinerea* (6) *Russula compacta* (6) *Russula fragilis* (2) *Amanita flavoconia* (2) Dermateaceae sp. (2) *Sistotrema* sp. (2) Helotiaceae sp. III (1) *Piloderma sphaerosporum* (1)	*Cortinarius* sp. (17) *Leotia lubrica* (3) *Piloderma fallax* (2) *Piloderma sphaerosporum* (2) *Penicillium spinulosum* (2) *Cortinarius jughuhnii* (2) *Cryptococcus* sp. (1) Dermateaceae sp. (1) *Hebeloma velutipes* (1) Helotiaceae sp. III (1) *Meliniomyces variabilis* (1) *Rhizoscyphus ericae* (1)
ML	*Piloderma* sp. (7) *Truncocolumella* sp. (6) *Lactarius tabidus* (6) *Lactarius deceptivus* (2) *Lactarius chrysorrheus* (1) *Cenococcum geophilum* (4) Helotiaceae sp. VI (2) *Hyphodiscus* sp. (2) *Cortinarius* sp. (1) *Chloridium* sp. (1) Mytilinidaceae sp. (1)	*Piloderma* sp. (13) *Tomentella sublilacina* (5) *Cenococcum geophilum* (4) *Clavulina cinerea* (4) *Tylospora* sp. (3) *Russula silvestris* (2) *Trechispora* sp. (2) *Cortinarius armillatus* (1) *Penicillium spinulosum* (1) Herpotrichellaceae sp. (1)