

**PATTERNS AND MECHANISMS OF TREE SPECIES DIVERSITY
EFFECTS ON FINE ROOT PROCESSES ASSOCIATED WITH STAND
DEVELOPMENT IN BOREAL FORESTS**

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ABSTRACT

Over the last two decades, one major advance in ecology has been the demonstration that biodiversity has positive effects on a broad range of ecosystem functions. However, diversity-ecosystem functioning studies for belowground are underrepresented, due to methodological limitations and the relative inaccessibility to root systems. This lack of understanding of belowground processes has cast doubt on the predictability of various ecosystem models; the forecasting of which serve as the basis for numerous global policies. The objective of this dissertation, therefore, is to improve the understanding of patterns and mechanisms of tree species diversity effects on fine root processes associated with stand development in natural forest ecosystems. To achieve this goal, I initially conducted a global meta-analysis on the effects of species diversity on fine root productivity in diverse ecosystems by synthesizing the results of 48 published studies. This meta-analysis demonstrated a positive mixture effects on fine root biomass and production, and showed that the mixture effects increased with species richness across all ecosystem types. More importantly, the meta-analysis also revealed shifts in diversity effects over time in both forests and grasslands.

Inspired by the results of the meta-analysis, I conducted an empirical diversity experiment in the central region of the North American natural boreal forest, to examine the temporal (seasonal and developmental) changes in fine root production, and their underlying mechanisms associated with tree species diversity. I found that annual fine root production was higher in mixtures than the mean of single species dominated stands in all age classes, with a significantly higher magnitude of effects in mature than young stands. My results also indicated that the increased positive diversity effects with stand development was the result of multiple mechanisms, including higher horizontal soil volume filling, a thicker forest floor layer for rooting, a higher magnitude of complementarity in deep nutrient-poor soil layers, and stronger nutrient foraging toward soil layers with high nutrient concentrations in older than younger stands.

Whether the results obtained on productivity can be generalized to other ecosystem processes remains patchy. I therefore examined species mixture effects on fine root turnover and mortality along stand development. I found that like biomass production, fine root turnover and mortality were also higher in mixtures than the mean of single-species-dominated stands in all age classes, with a higher mixture effects in mature than young stands. Moreover, my results

suggested that increased mixture effects with stand development resulted from a higher competition intensity that was induced by the overyielding of fine root biomass production in mixtures.

Moreover, most published diversity and productivity relationship (DPR) studies focus on one component of ecosystem production. Species diversity could alter production allocation, at least, in part, contributing to divergent DPR relationships. By synthesizing the production data of all individual components (i.e., aboveground trees, litterfall, understory vegetation, coarse roots, and fine roots) of boreal forest stands, collected from the same study sites, I examined how species mixtures affected the production of the entire ecosystem, and production partitioning among individual components along stand development. I found that the overyielding of the entire ecosystem production occurred in young stands, but not in older stands, despite the fact that fine root production was higher in species mixtures than single-species dominated stands in all ages. Species mixtures led to more production allocated to belowground than expected from single species-dominant stands.

These studies offer a new and important understanding of DPR by showing the temporal changes of mixture effects on fine root dynamics (i.e., production, turnover, and mortality), production allocation, and their underlying mechanisms. The results have relevance for calculating the energy allocation, as well as the carbon storage of terrestrial ecosystems, and may provide a broad guide for management practices with the aim of increasing belowground productivity, element cycling, and carbon sequestration.

Key-words: Biodiversity and ecosystem functions, carbon storage, fine root process, mixture effects, meta-analysis, mechanisms, natural boreal forest, nutrient availability, overyielding, production, production partitioning, root interaction, stand development, turnover and mortality.

CONTENTS

| | |
|---|----|
| ABSTRACT..... | ii |
| CONTENTS..... | iv |
| CHAPTER 1: GENERAL INTRODUCTION | 1 |
| CHAPTER 2: SPECIES DIVERSITY EFFECTS ON FINE ROOT PRODUCTIVITY IN DIVERSITY ECOSYSTEMS: A GLOBAL META-ANALYSIS..... | 3 |
| 2.1 Abstract..... | 3 |
| 2.2 Introduction..... | 4 |
| 2.3 Material and methods..... | 7 |
| 2.3.1 Data collection | 7 |
| 2.3.2 Data analysis | 8 |
| 2.4 Results..... | 12 |
| 2.5 Discussion..... | 18 |
| CHAPTER 3: EFFECTS OF SPECIES DIVERSITY ON FINE ROOT PRODUCTIVITY INCREASE WITH STAND DEVELOPMENT AND ASSOCIATED MECHANISMS IN A BOREAL FOREST..... | 21 |
| 3.1 Abstract..... | 21 |
| 3.2 Introduction..... | 22 |
| 3.3 Materials and methods | 25 |
| 3.3.1 Study area..... | 25 |
| 3.3.2 Sampling design..... | 26 |
| 3.3.3 Data collection | 27 |
| 3.3.4 Statistical analysis..... | 30 |

| | |
|---|----|
| 3.4 Results..... | 32 |
| 3.5 Discussion..... | 39 |
| CHAPTER 4: POSITIVE SPECIES MIXTURE EFFECTS ON FINE ROOT TURNOVER AND MORTALITY INCREASE WITH STAND DEVELOPMENT IN NATURAL BOREAL FORESTS | |
| 4.1 Abstract..... | 44 |
| 4.2 Introduction..... | 45 |
| 4.3 Material and methods..... | 49 |
| 4.3.1 Study area and experimental design | 49 |
| 4.3.2 Data collection | 50 |
| 4.3.3 Statistical analysis..... | 52 |
| 4.4 Results..... | 54 |
| 4.5 Discussion..... | 57 |
| 4.6 Conclusion | 63 |
| CHAPTER 5: EFFECT OF SPECIES MIXTURES ON PRODUCTION PARTITIONING ALONG STAND DEVELOPMENT IN A NATURAL BOREAL FOREST..... | |
| 5.1 Abstract..... | 64 |
| 5.2 Introduction..... | 65 |
| 5.3 Materials and methods | 69 |
| 5.3.1 Study area and experimental design | 69 |
| 5.3.2 Data collection | 70 |
| 5.3.3 Statistical analysis..... | 74 |
| 5.4 Results..... | 75 |

| | |
|---|-----|
| 5.5 Discussion | 79 |
| 5.6 Conclusion | 86 |
| CHAPTER 6: GENERAL CONCLUSION..... | 87 |
| REFERENCES | 89 |
| APPENDIX I. SUPPLEMENTAL INFORMATION FOR CHAPTER 2 | 115 |
| APPENDIX II. SUPPLEMENTAL INFORMATION FOR CHAPTER 5 | 121 |

LIST OF TABLES

| | |
|--|----|
| Table 2.1. Natural log-transformed response ratio ($\ln RR$) in relation to predictors determined by weighted (w_{rn}) multiple regression. Coefficients were bootstrapped with 4,999 iterations. Bivariate relationships are represented by the coefficients of simple or quadratic regression. Variables listed in boldface indicate significant effects ($P < 0.05$). The number in parentheses represents bootstrapped 95% confidence interval. $\ln(S)$, $\ln(\text{Exp age})$, R^2 , and P are natural log transformed species richness, natural log transformed experiment age, explained variance by the model, and significance of the model, respectively. Cropland was not included as there is no variation in species richness in mixtures..... | 17 |
| Table 3.1. Characteristics (mean and 1 s.e.m., $n = 3$) of the study stands in Northwestern Ontario, Canada. Stand types are single-species <i>Pinus banksiana</i> dominated (<i>Pinus</i>), single-species <i>Populus tremuloides</i> dominated (<i>Populus</i>), and their mixtures (<i>Populus + Pinus</i>). | 29 |
| Table 3.2. Effect of stand age on mixture effect. Mixture effects represent by R_o (eqn. 1) and R_t (eqn. 3) | 33 |
| Table 3.3. Effects of stand type and age on annual fine root production. | 34 |
| Table 3.4. Effects of stand age and sampling date on fine root biomass overyielding (R_o , eqn. 1) and transgressive overyielding (R_t , eqn. 3). | 35 |
| Table 3.5. Effects of stand type, age and sampling date on fine root biomass and homogeneity index. | 35 |
| Table 3.6. Effects of stand age and sampling date on horizontal homogeneity index of fine root biomass (R_o , eqn. 1) and (R_t , eqn. 3). | 37 |

| | |
|--|----|
| Table 3.7. Effects of stand age and type on the relationship between fine root production per soil depth and nutrient concentration. N and P means nitrogen and phosphorus concentration, respectively. | 41 |
| Table 4.1. Shannon's index, forest floor depth and understorey vegetation cover (mean and 1 s.e.m., $n = 3$) of the study stands. Stand types are single-species <i>Pinus banksiana</i> dominated (<i>Pinus</i>), single-species <i>Populus tremuloides</i> dominated (<i>Populus</i>), and their mixtures (<i>Populus + Pinus</i>). | 51 |
| Table 4.2. Effects of stand age on R_o and R_t for fine root turnover and mortality. | 55 |
| Table 4.3. Effects of stand type and age on fine root turnover and mortality. | 56 |
| Table 4.4. Effects of tree species richness, evenness, and Shannon's diversity index (H) on fine root turnover and mortality of the entire soil profile among stand ages. | 60 |
| Table 5.1. Classification of five components of production ($\text{Mg ha}^{-1} \text{ year}^{-1}$) in natural forests. | 66 |
| Table 5.2. The associations between soil nutrients and the first two PCA axes. The two PCA axes accounted for a total of 92.5% of the variation in soil nutrients (79.9% and 12.5%, respectively)..... | 79 |

LIST OF FIGURES

Figure 2.1. Comparison of fine root biomass between species mixtures and monocultures in various ecosystem types. (a-c) Overyielding of fine root biomass by different weighting functions; (d-f) Transgressive overyielding of fine root biomass by different weighting functions. Wr_n , Wr_r , and Wu are weights by replications divided by number of observations, replications, and unweighted, respectively. Results (bootstrapped mean and 95% confidence intervals of effect size) are shown for the entire data set (overall) and for forests, natural forests, planted forests, grasslands, croplands, and pot systems. The number of studies and observations of each ecosystem type is displayed in parentheses. 13

Figure 2.2. The response of fine root production to species mixtures and its relationship with fine root biomass. Results (bootstrapped mean and 95% confidence intervals of effect size) are shown for overall and by natural forests, planted forests, and grasslands. Wr_n , Wr_r and Wu are weighting functions described in Figure 2.1. The number of studies and observations of each ecosystem type is displayed in parentheses. (d) The relationship between the natural log response ratios ($\ln RR$) of fine root production and biomass. 14

Figure 2.3. Determinants of diversity effects on fine root biomass. Partial regression plots show the natural log response ratios ($\ln RR$) of fine root biomass in mixtures to that in monocultures in relation to (a) richness, (b) stand age, and (c) experiment age from weighted bootstrapping multiple linear regression models (Table 2.1) once all other variables in the model are statistically controlled for. Black lines represent overall response across all ecosystem types (Table 2.1). Colors indicate ecosystem types: reddish purple—natural forest ($n = 35$), sky blue—planted forest ($n = 34$), grey—grassland ($n =$

76), bluish green—cropland ($n = 10$), and vermilion—pot ($n = 15$). The sizes of circles represent the relative weights of corresponding observations. Solid lines represent significant relationships ($P < 0.05$), and dashed lines insignificant ($P \geq 0.05$)..... 16

Figure 3.1. Potential mechanisms for temporal change of diversity effects on fine root productivity in forest ecosystems. FF and MS are forest floor and mineral soil, respectively. 25

Figure 3.2. Effects of mixture on annual fine root production in relation to stand development. Mean and 95% confidence intervals of R_o and R_t higher than 1 indicate overyielding and transgressive overyielding of annual fine root production, respectively. Stand types were single-species *Populus tremuloides* dominated (*Populus*), single-species *Pinus banksiana* dominated (*Pinus*), and their even mixture (*Populus + Pinus*). Error bars represent 1 s.e.m. ($n = 3$). 33

Figure 3.3. Effects of mixture on fine root biomass and homogeneity index along sampling date for each stand age. (a, c) 8-year-old stands, (b, d) 34-year-old stands. Mean and 95% confidence intervals of R_o and R_t higher than 1 indicate overyielding and transgressive overyielding of fine root biomass or homogeneity index, respectively. Stand types are described in Figure 3.2. Error bars represent 1 s.e.m. ($n = 3$). 36

Figure 3.4. Spatial variation of fine root biomass (box plots) and corresponding horizontal homogeneity index (circles and lines) in relation to the sampling date for each stand type and age. Box plots show the median (line within the box), 25th and 75th percentiles (the boundaries of the box) and 90th and 10th percentiles (error bars) of mean fine root biomass of seven soil cores, which were ranked from large to small within each plot, of the three

| | |
|--|----|
| replicate plots. (a) 8-year-old stands, (b) 34-year-old stands. Error bars represent 1 s.e.m. (n = 3). | 38 |
| Figure 3.5. Relationship between natural log transformed fine root biomass and homogeneity index. Type II regression were used to fit the model..... | 37 |
| Figure 3.6. Annual fine root production in relation to soil layers. (a, b) Annual fine root production by stand type (Error bars represent 1 s.e.m., n = 3) and mixture effects (calculated as overyielding, Ro mean and 95% confidence intervals) in 8-year-old and 34-year-old stands. Relationships between annual fine root production per soil depth and N concentrations (c, d) and P concentrations (e, f) in 8-year-old and 34-year-old stands, respectively. Colors indicate soil layers: vermilion—Forest floor, sky blue—MS1, and reddish purple—MS2. Shapes indicate stand type: circle—Populus stands, triangle—Pinus stands, and square—Populus + Pinus stands. Fitted lines represent significant relationships (P < 0.05). Dots and error bars represent mean values and 1 s.e.m. (n = 3). | 40 |
| Figure 4.1. Effects of mixture on fine root turnover (a, c) and mortality (b, d) in relation to stand development. Mean and 95% confidence intervals of Ro and Ri higher than 1 indicate overyielding and transgressive overyielding of fine root turnover or mortality, respectively. Stand types were single-species <i>Populus tremuloides</i> -dominated (<i>Populus</i>), single-species <i>Pinus banksiana</i> -dominated (<i>Pinus</i>) and their even mixture (<i>Populus</i> + <i>Pinus</i>). Error bar represent 1 SEM (n = 3). Different letters indicate a significant difference between stands within the same age category (α = 0.05). | 55 |
| Figure 4.2. Effects of mixture on fine root turnover and mortality in relation to soil layers for each stand age. Turnover rates and mortality were standardized by soil depth on per cm | |

basis due to variable depths of forest floor layer. Soil layers are forest floor (FF), mineral layer 1 (MS1, 0-15 cm) and mineral layer 2 (MS2, 15-30 cm). Mixture effects were calculated as overyielding, R_o mean and 95% confidence intervals. Stand types were described in Figure. 1. Error bar represent 1 SEM ($n = 3$). 56

Figure 4.3. Fine root turnover (a-c) and mortality (d-f) in relation to tree species richness, evenness, and Shannon’s diversity index. 8- and 34-year-stands were represented by red and blue circles, respectively. Black lines represent overall response across two stand ages ($n = 18$). Solid lines represent significant relationships ($P < 0.05$), and dashed lines insignificant ($P \geq 0.05$). 58

Figure 4.4. Pattern of increasing turnover rates and mortality with greater fine root production (a, c) and average standing fine root biomass (b, d) observed across stand types and ages. 8- and 34-year-stands were represented by open and closed shapes, respectively. Within each panel, the solid line represents a significant relationship through all three stand types and ages ($n = 18$), while the dashed lines represent an insignificant relationship. Reported P value and R^2 are shown for regression lines across all stand types. 59

Figure 4.5. Effects of mixture on fine root turnover (a, c) and mortality (b, d) in relation to stand development based on MaxMin method. Mean and 95% confidence intervals of R_o and R_t higher than 1 indicate overyielding and transgressive overyielding of fine root turnover or mortality, respectively. Stand types were described in Figure. 1. Error bar represent 1 SEM ($n = 3$). 61

Figure 5.1. Effects of mixture on production of each individual component and entire ecosystem (upper panel), expected and observed values of production for the mixtures (lower panel), in relation to stand development. Mixture effects are represented by scatter with mean

and 95% confidence intervals. Dark green: aboveground trees; light blue: understorey vegetation; light green: litter fall; vermilion: coarse roots; orange: fine roots; black: entire ecosystem. Error bar represent 1 SEM ($n = 3$). 76

Figure 5.2. Effects of mixtures on production fraction of each individual component, and total belowground (upper panel), expected and observed values of production fraction for the mixtures (lower panel) in relation to stand development. Mixture effects are represented by scatter with mean and 95% confidence intervals. Dark green: aboveground trees; light blue: understorey vegetation; light green: litter fall; vermilion: coarse roots; orange: fine roots; black: total belowground. Error bar represent 1 SEM ($n = 3$). 77

Figure 5.3. The first two principal components of soil nutrients change with stand development. Error bar represent 1 SEM ($n = 3$). 79

Figure 5.4. Partial regression plots show the production allocation in relation to the first two principal components of soil nutrients (PC1 and PC2). Colours indicate stand age classes: red, 8 years; green, 34 years; blue, 85 years. Within each panel, the solid line represents a significant relationship through all three stand types and ages ($n = 27$), while the dashed lines represent an insignificant relationship. 81

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CHAPTER 1: GENERAL INTRODUCTION

In recent decades, the biodiversity and ecosystem functioning relationship (BEF) has been a major ecological research focus to help understand the impact of global species extinction crisis on ecosystem functioning (Cardinale et al. 2012). The relationships between diversity and above-ground productivity have been well studied, and positive relationships have been observed across different ecosystem types (Tilman et al. 1996, Loreau and Hector 2001, Cardinale et al. 2007, Liang et al. 2016). For many biomes, plant biomass production is allocated to below-ground more than above-ground (Reich et al. 2004, Poorter et al. 2012), with fine root ($\varnothing \leq 2$ mm) production alone accounting for 22% of the total annual net primary production of global terrestrial ecosystems (McCormack et al. 2015). In addition, fine roots with high turnover and mortality rates are a major contributor to nutrient cycling and carbon accumulation, translocating carbon and nutrients from roots to the long-lasting soil organic pool (Richter et al. 1999, Tefs and Gleixner 2012). In the boreal forest, approximately 50 to 70% of soil carbon results from the mortality of roots and root-associated microorganisms (Clemmensen et al. 2013). Despite this, diversity-ecosystem functioning studies for below-ground are under-represented, particularly in natural forests.

In addition, the ecosystem functions and services provided by forests reflect the contributions of all components. However, the vast majority of diversity and productivity relationship studies have come from experiments that separately tested diversity effects on a certain component of production (e.g., aboveground biomass and fine roots) (Zhang et al. 2012, Ma and Chen 2016). How productivity responds to diversity from the whole ecosystem perspective, and how mixtures affect production partitioning among individual components remains unknown.

The objective of this dissertation is to improve the understanding of patterns and mechanisms of tree species diversity effects on fine root processes associated with stand development in natural forest ecosystems. To achieve this goal, I first conducted a global meta-analysis on the effects of species diversity on fine root productivity in diverse ecosystems by synthesizing 48 published studies. Inspired by the result of this meta-analysis, I conducted an empirical diversity experiment in the central region of North American natural boreal forest, to examine the temporal (seasonal and developmental) changes in fine root production, and their underlying mechanisms associated with tree species diversity. Next, I tested whether the results obtained on productivity can be generalized to other ecosystem processes, such as root turnover and mortality. Lastly, by synthesizing the production data of all individual components (i.e., aboveground trees, litterfall, understory vegetation, coarse roots, and fine roots) of boreal forest stands, collected from same study sites, I examined how species mixture affected the production of entire ecosystems and production partitioning among individual components along stand development.

Chapters 2 and 3 of this dissertation have already been published (Chapter 2 in *Global Ecology and Biogeography* (Ma & Chen 2016) and Chapter 3 in *Journal of Ecology* (Ma & Chen 2017)). Chapter 4 is currently under review by *Functional Ecology*. Since each chapter has been written as a distinct manuscript to facilitate publication, I have made reference to Chapter 2 in Chapters 3, 4, and 5; and Chapter 3, in Chapters 4 and 5, respectively. Also, since individual chapters (published, in review, or to be submitted) reflect joint contributions from myself and my academic supervisor, I presented “we” instead of “I” as is written for individual manuscripts.

CHAPTER 2: SPECIES DIVERSITY EFFECTS ON FINE ROOT PRODUCTIVITY IN DIVERSITY ECOSYSTEMS: A GLOBAL META- ANALYSIS

2.1 Abstract

Positive relationships between plant species diversity and above-ground productivity have been observed across a wide range of terrestrial ecosystems. Despite a critical contribution of below-ground productivity to overall terrestrial productivity, no consensus exists about the nature of the relationship between species diversity and below-ground productivity. We collected data from published studies conducted in natural and planted forests, and experimental grassland, crop, and pot systems that were purposely implemented to isolate the effects of plant species diversity from other factors, such as soil conditions and topographic features. We conducted a meta-analysis of 170 observations for root biomass and 23 observations for root production derived from 48 published studies using weighted linear model with bootstrap procedures to reconcile diversity effects on fine root productivity. We found that species mixtures had, on average 28.4% higher fine root biomass and 44.8% higher annual production than monocultures. Higher fine root biomass in species mixtures than monocultures was consistent across natural forests, planted grasslands, croplands, and pot systems except for young planted forests. Transgressive overyielding was evident only for planted grasslands. The log response ratio of fine root biomass in species mixtures to that in respective monocultures increased with species richness across all ecosystem types, and also increased with experiment age in grasslands. Our meta-analysis reveals positive effects of

species diversity on belowground productivity. Despite profound differences in environments among terrestrial ecosystems, our analysis demonstrated that belowground productivity responds similarly to the variations in species richness. Furthermore, our study also reveals shifts in diversity effects over time in both forests and grasslands. Future efforts are needed to further understand below-ground productivity and diversity relationships.

2.2 Introduction

During the past two decades, one of the major advances in ecology has been demonstrating that biodiversity has positive effects on a wide range of ecosystem functions, particularly on annual net primary production (ANPP) (Hooper et al. 2005, Hooper et al. 2012, Naeem et al. 2012a). The relationships between diversity and above-ground productivity have been well studied, and two recent meta-analyses have revealed that mixtures generally have higher productivity than monocultures (Cardinale et al. 2007, Zhang et al. 2012). For many biomes, plant biomass production is allocated to below-ground more than above-ground (Reich et al. 2004, Poorter et al. 2012), with fine root ($\varnothing \leq 2$ mm) production alone accounting for 22% of the total ANPP of global terrestrial ecosystems (McCormack et al. 2015). Despite this, diversity-ecosystem functioning studies for below-ground productivity are under-represented. This is likely due to methodological limitations and the relative inaccessibility of root systems (Hendricks et al. 2006, Brassard et al. 2013). This lack of understanding of below-ground processes has cast doubt on the predictability of various ecosystem models (Jackson et al. 2000), the forecasts of which serve as the basis for numerous global policies (IPCC 2013).

A positive effect of diversity on fine root productivity has recently been reported, i.e., ‘overyielding’ in species mixtures than monocultures (Mommer et al. 2010, Brassard et al. 2013, Lang'at et al. 2013, Jacob et al. 2014). Several mechanisms have been proposed to

explain this phenomenon. Mixed species with trait differences in rooting depth, root system architectures and/or root foraging behaviour allow them to exploit diverse below-ground niches, such as different soil depths, which may result in greater overall resource uptake and productivity (Dimitrakopoulos and Schmid 2004, Silvertown 2004, Stubbs and Wilson 2004). Root growth can be stimulated by the presence of heterospecific root neighbours (Schenk 2006, de Kroon 2007, Mommer et al. 2010) and interspecific root interactions may result in increased biomass by growing comparably more roots (Brassard et al. 2013, Mueller et al. 2013). The lower pathogenic pressures compared with monocultures could be another reason for increased fine root biomass in mixtures (Maron et al. 2011, de Kroon et al. 2012). However, evidence for below-ground overyielding remains controversial, with reports of positive (Reich et al. 2004, Brassard et al. 2011b, Brassard et al. 2013, Mueller et al. 2013), neutral (Meinen et al. 2009a, Meinen et al. 2009c, Lei et al. 2012b, Xiang et al. 2013, Domisch et al. 2015) and even negative effects (Bolte and Villanueva 2006b). The causes of these various results may be attributable to the selection of ecosystem types, the range of species diversity investigated, and sample sizes. Furthermore, divergent findings may arise because of the temporal variation in diversity effects (Tilman et al. 2001, Reich et al. 2012, Zhang et al. 2012, Turnbull et al. 2013).

Diversity effects on fine root productivity may differ among ecosystem types (Forrester and Bauhus 2016), and between natural and planted systems. For example, positive diversity effects may be stronger in resource limited grasslands than forests since the strength of positive species interactions tend to increase with environmental stress (Goldberg et al. 1999, Maestre et al. 2009). While experiments under controlled homogeneous environments, such as experimental plantations, allow for a mechanistic understanding of diversity effects,

the positive diversity effects under controlled experiments may not reflect the processes in heterogeneous natural environments (Adler et al. 2011, Naeem et al. 2012b). Alternatively, since resource heterogeneity in natural environments can increase the realized effects of niche complementarity (Stachowicz et al. 2008), greater diversity effects on productivity may occur in natural rather than controlled systems.

Temporal changes of diversity effects on above-ground productivity have been previously reported (Zhang et al. 2012). In a long-term grassland experiment, Tilman et al. (2001) first found that the effect of species richness on aboveground biomass became progressively stronger over time. In forest ecosystems, effects of plant species diversity were found to change with successional stage being investigated (Zhang et al. 2012, Lasky et al. 2014). Potential reason for these temporal change of diversity effects may be attributed to increasing species complementarity over time (Cardinale et al. 2007) or fading of functional redundancy through time (Reich et al. 2012). However, current knowledge is weak regarding the potential temporal change of diversity effects on below-ground productivity. We predict that diversity effects on fine root productivity will increase over time.

Here, we compiled data from 48 studies to examine the effect size of species diversity, measured as the ratio of the fine root productivity and biomass in mixtures to the average of those in monocultures as well as that in most productive monocultures. We specifically tested whether 1) mixtures overyield monocultures for fine root productivity across a wide range of terrestrial ecosystem types; 2) the effect size increases with species richness, stand or experiment age.

2.3 Material and methods

2.3.1 Data collection

We searched all peer-reviewed publications that investigated the effects of diversity on fine root productivity using ISI Web of Science, Forest Science Database and Google Scholar up to June 1, 2015. Different keyword combinations, such as fine root, biomass, production, diversity, mixture, pure, polyculture, monoculture, richness, and evenness were used for the search. The following criteria were applied to select the appropriate observations: (i) studies were purposely implemented to isolate the effects of plant species diversity from other factors, such as soil conditions and topographic features of sampling plots; (ii) mean fine root biomass and/or production could be extracted directly from text, tables and/or figures. These criteria resulted in 40 publications that encompassed forests, grasslands, croplands, and pots. Of the 40 publications, six reported both fine root biomass and production. The methods used for biomass and production estimates were all by harvesting through soil cores or pits (one time or sequential over a growing season or ingrowth cores) except one by N¹⁵ natural abundance method. When the same data were reported in different publications, the data were only recorded once in our meta data. A list of data sources is found in [Appendix 1](#). In some publications where several experiments, each with their independent control, were done in different locations or under different abiotic treatments, we considered them as different comparisons (studies). For studies which include multiple non-independent observations caused by soil layers, we summed biomass of all layers to obtain total fine root biomass or annual production for entire soil profile sampled (Hungate et al. 2009). This resulted in a total of 48 studies and 170 observations. Among them, 19 studies had single observation. In forests, studies were categorized as natural or planted origin, whereas studies in other systems

were all planted origin. When an original study reported results graphically, we utilized SigmaScanPro version 5 (Systat Software Inc., Point Richmond, CA, USA) to digitally extract data from figures.

For each study, we extracted data on fine root biomass, annual fine root production (if available), geographical location, ecosystem type and origin, species richness, experiment age (years) in grasslands and stand age (years) in forests from the original papers. Experiment age in grasslands was the number of years between experiment initiation and biomass harvests. Stand age was determined based on site descriptions of the original studies. Sample size corresponding to each observation was derived based on the number of independent experimental units or sampling sites.

2.3.2 Data analysis

We used the natural log-transformed response ratio ($\ln RR$) as the effect size, which improves its statistical behaviour in meta-analyses (Hedges et al. 1999), as an index to examine the responses of fine root biomass and annual production.

$$\ln RR = \ln(\bar{X}_t / \bar{X}_c) = \ln \bar{X}_t - \ln \bar{X}_c \quad (1)$$

where \bar{X}_t and \bar{X}_c are the observed fine root biomass or annual production in mixtures and the mean fine root biomass or annual production of all monocultures in each study, respectively. When a study reported multiple types of mixtures, experiment age, and stand age, \bar{X}_t and \bar{X}_c were calculated separately for each mixture type, experiment age and stand age. In order to test if “transgressive” overyielding occurs (i.e., if root biomass in mixtures is higher than the most productive monoculture), \bar{X}_c in the above formula was replaced by fine root biomass in the most productive monoculture (\bar{X}_b). We did not test “transgressive”

overyielding for production because there were only three publications reported fine root production of individual monocultures. Monocultures were pure single-species stands in 46 of the 48 studies. In two studies where single species stands were not available, monocultures were defined by single species that comprised $\geq 80\%$ of stand basal areas, as in the original studies (Brassard et al. 2011b).

Because effect size estimates and subsequent inferences in meta-analysis may depend on how individual observations are weighted (van Groenigen et al. 2011, Mueller et al. 2012), we compared the influences of six weighting functions on effect size estimates. These weights for observations included (i) by sampling variance (w_s) (Eqn. 2) (Hedges et al. 1999), (ii) by sampling variance divided by the total number of observations of each study to account for the non-independence of the observations within each study (w_{sn}) (Eqn. 3) (Pittelkow et al. 2015), (Chapin III et al.) by the number of replications (w_r) (Eqn. 4), (iv) by the number of replications divided by the total number of observations from each study (w_{rn}) (Eqn. 5), (v) by the inverse of number of observations from each study ($w_{1/n}$) (Eqn. 6) and (vi) by an equal weigh for each: $w_u = 1$.

$$w_s = 1 / \left(\frac{S_t^2}{n_t \bar{X}_t^2} + \frac{S_c^2}{n_c \bar{X}_c^2} \right) \quad (2)$$

$$w_{sn} = 1 / \left(\frac{S_t^2}{n_t \bar{X}_t^2} + \frac{S_c^2}{n_c \bar{X}_c^2} \right) \times \frac{1}{N} \quad (3)$$

$$w_r = \frac{n_t \times n_c}{n_t + n_c} \quad (4)$$

$$w_{rn} = \frac{n_t \times n_c}{n_t + n_c} \times \frac{1}{N} \quad (5)$$

$$w_{1/n} = \frac{1}{N} \quad (6)$$

where S_t , n_t , S_c , and n_c are the standard deviation and sample size for the mixture and monocultures, respectively. N is the total number of observations of each study. Four studies did not report standard deviations, hence we calculated the average coefficient of variation (CV) within each data set, and then approximated the missing standard deviation by multiplying the reported mean by the average CV (Bai et al. 2013).

Results using the different weighting functions like w_r , w_{rn} , $w_{1/n}$, and w_u which yielded weights that varied over 78, 60, 25 and 1 times in magnitude, respectively, were qualitatively similar (Appendices 2 and 3). However, weighting functions like w_s and w_{sn} , which varied over 4,900 and 20,000 times in magnitude, respectively, gave slightly different results. By assigning extreme importance to individual observations, average effect sizes were largely determined by a small number of studies. Moreover, because variance estimates are notoriously unreliable (especially given the small samples common in many of these studies) and large variances could have resulted from sampling from diverse site conditions, we favoured the use of the alternative weighting function w_{rn} (which assigned less extreme weights and gave less weights to studies with multiple non-independent observations). We focus on reporting the results based on weighting by w_{rn} .

Although fine roots turn over rapidly, varying with species traits (Yuan and Chen 2010), the extent of fine root biomass reflects fine root productivity is unclear. Based on those studies that reported both biomass and annual production, we examined the relationship between biomass and production by using type II regression, which is suited for relationships when variables cannot be clearly distinguished as independent or dependent, and when error is associated with the measures of both. To examine how the variation in species richness,

experiment age (only available in grassland experiments) and stand age (only available in forest ecosystems) affected $\ln RR$, we examined their bivariate relationships. To assess potential nonlinearity between $\ln RR$ and species richness, experiment age, and stand age, we compared linear, quadratic, and logarithmic functions for each of these continuous variables and selected the best bivariate relationships based on Akaike Information Criterion (Litton et al.): the simpler model was selected when the difference in AICs between alternative models was <2 (Burnham and Anderson 2002). These bivariate relationships were examined by linear models weighted by w_{rn} .

Since our data reflect multiple drivers on $\ln RR$, we used multiple regression analysis, weighted by w_{rn} , to examine the responses of $\ln RR$ to species richness, experiment age and stand age (where applicable). The above analyses are parametric and assume normally distributed data and homogeneous variances. However, these assumptions were violated based on Shapiro-Wilk's test and Breusch-Pagan test, respectively. Thus, we bootstrapped the estimates of $\ln RR$ and regression coefficients by using the '*boot*' package (Canty and Ripley 2012) with 4,999 iterations to generate 95% confidence intervals (CIs) (Adams et al. 1997). When the 95% CIs does not cover zero, $\ln RR$ or regression coefficient is significantly different from zero. For ease of interpretation, mean $\ln RR$ and its corresponding confidence intervals were transformed back to the percentage change between pure and species mixtures:

$$(e^{\overline{\ln RR}} - 1) \times 100\% \tag{7}$$

All independent variables were centred without dividing by the standard deviation to facilitate coefficient interpretation. All statistical analyses were conducted with R (version 3.2.2).

2.4 Results

The overall mean effect size for fine root biomass, based on the weighting by w_{rn} , was 0.25 (95% bootstrapped confidence interval, CI, 0.15 – 0.35), indicating that mixtures, on average, had a 28.4% (CI, 16.2% – 41.9%) higher fine root biomass than monocultures (Figure 2.1). The response of fine root biomass to species mixture varied among ecosystem types with a significantly higher effect size in grasslands, which on average had higher species richness (Appendix 4). Although higher fine root biomass in mixtures than in monocultures were found in natural forests, grasslands, croplands and pots, significant overyielding of fine root biomass occurred only in grasslands and pots, with 64.9% and 12.7% higher fine root biomass in mixtures than in monocultures, respectively (Figure 2.1). When forests were separately analyzed for natural versus planted, diversity effect was positive in natural forests but negative in planted forests, however, both effects were not significant (Figure 2.1). The estimated effect sizes based on the weightings of w_r and w_u were qualitatively similar to those based on w_{rn} (Figure 2.1). Overall fine root biomass was on average 17.3% (CI, 5.1% – 31.0%) higher in mixture than that in most productive monocultures (Figure 2.1). When individual ecosystem types were considered, transgressive overyielding occurred only in grasslands (Figure 2.1).

Annual fine root production was on average 44.8% (CI, 29.7% – 61.6%) higher in mixtures than monocultures (Figure 2.2). Fine root production was higher in mixtures than in monocultures by 61.6% (CI, 41.9% – 84.0%) in grasslands and 31.0% (CI, 15.0% – 49.2%) in natural forests, whereas annual fine root production did not differ between mixtures and monocultures in planted forests (Figure 2.2). Based on the studies that reported

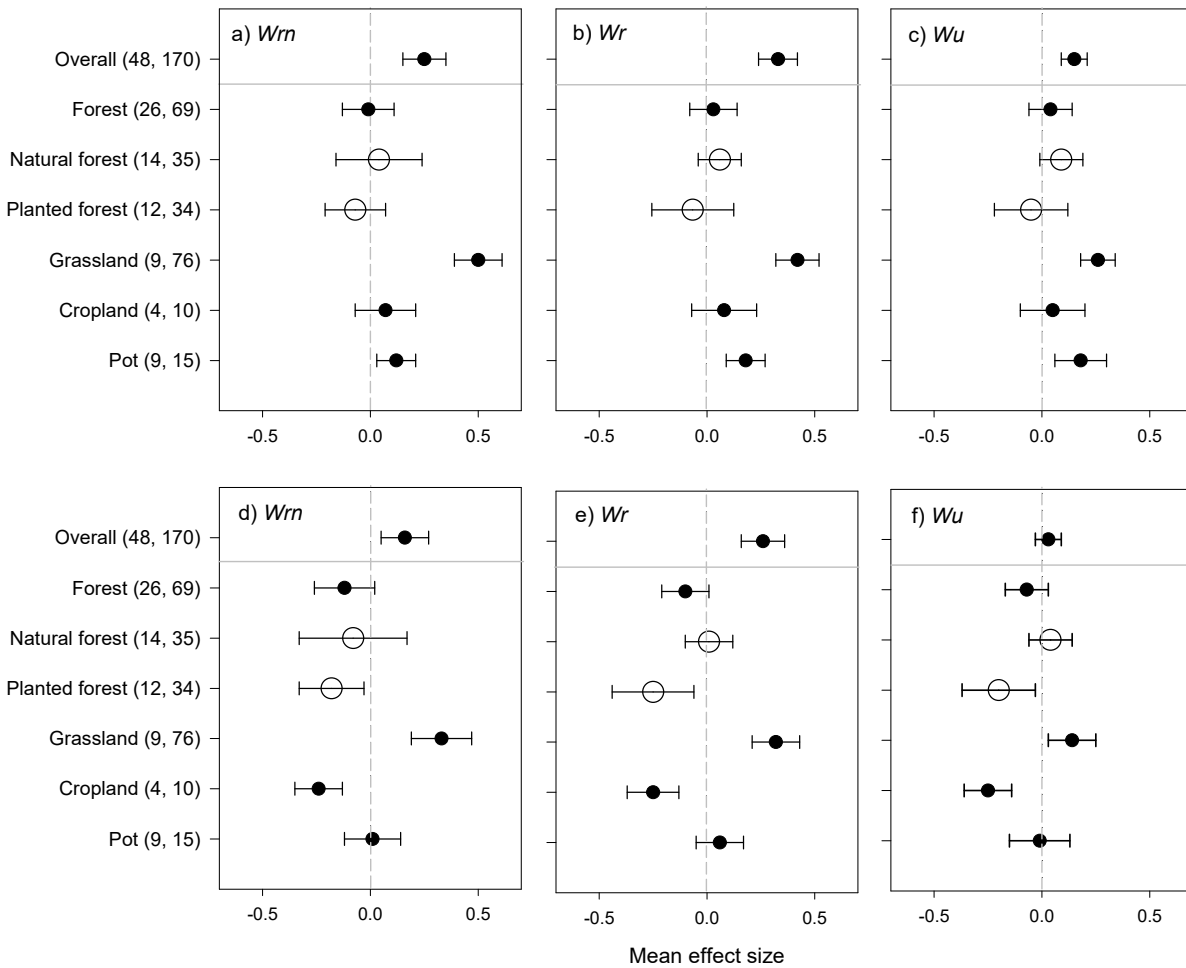


Figure 2.1. Comparison of fine root biomass between species mixtures and monocultures in various ecosystem types. (a-c) Overyielding of fine root biomass by different weighting functions; (d-f) Transgressive overyielding of fine root biomass by different weighting functions. W_{rn} , W_r , and W_u are weights by replications divided by number of observations, replications, and unweighted, respectively. Results (bootstrapped mean and 95% confidence intervals of effect size) are shown for the entire data set (overall) and for forests, natural forests, planted forests, grasslands, croplands, and pot systems. The number of studies and observations of each ecosystem type is displayed in parentheses.

both biomass and production, there was a positive correlation between the effect sizes of biomass and production ($r^2 = 0.64$, $P < 0.001$) (Figure 2.2).

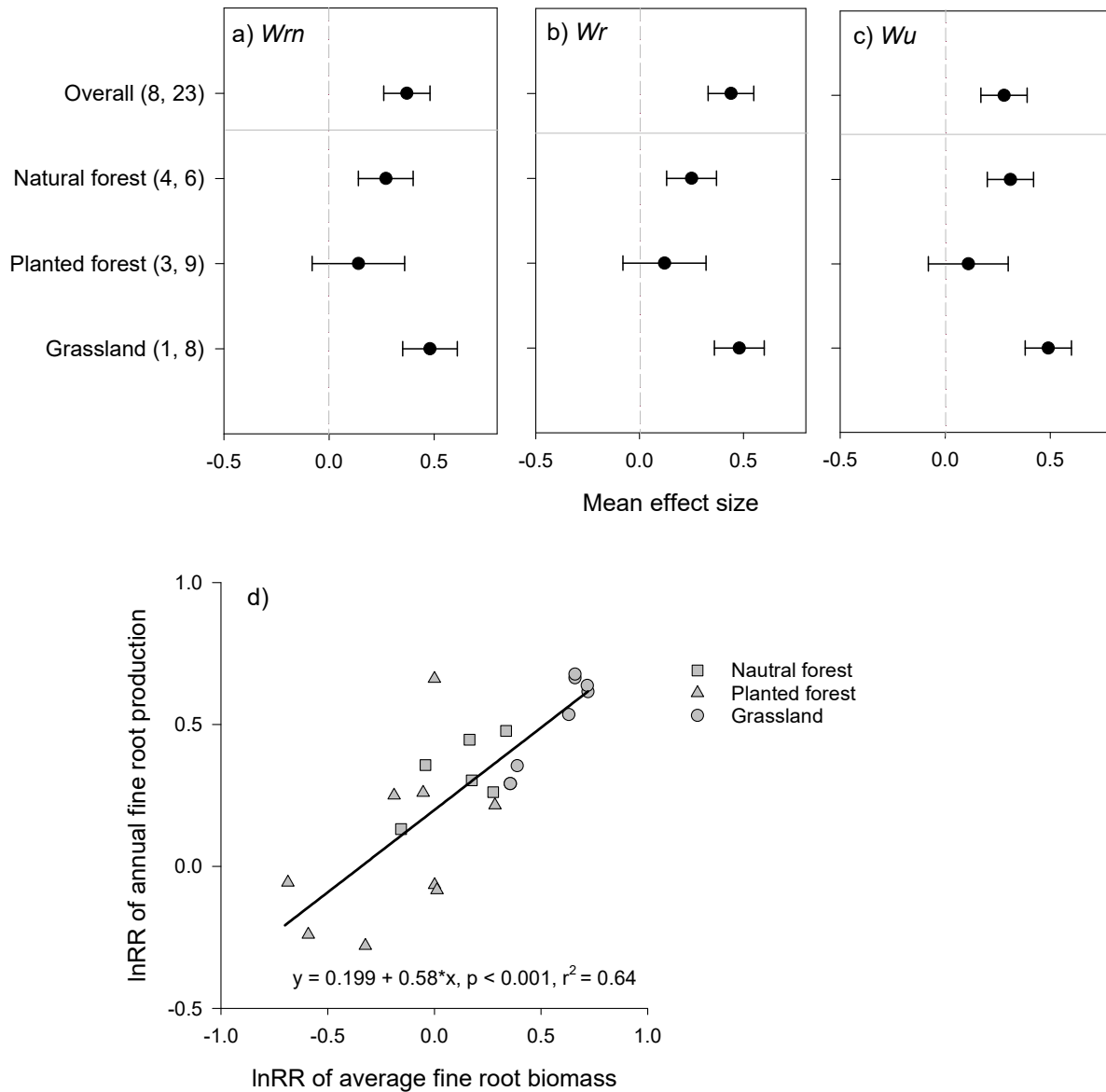


Figure 2.2. The response of fine root production to species mixtures and its relationship with fine root biomass. Results (bootstrapped mean and 95% confidence intervals of effect size) are shown for overall and by natural forests, planted forests, and grasslands. W_m , W_r and W_u are weighting functions described in Figure 2.1. The number of studies and observations of each ecosystem type is displayed in parentheses. (d) The relationship between the natural log response ratios (lnRR) of fine root production and biomass.

With all data pooled, effect size increased significantly with species richness (Table 2.1, Figure 2.3) and this positive effect did not differ significantly among ecosystem types (P

=0.12, Appendix 5). When individual ecosystem types were examined, significant positive effect of species richness on effect size were found in both natural forest and grasslands (Table 2.1, Figure 2.3). Effect size responded quadratically to stand age in forests with a larger effect size in the intermediately aged than young or old stands (Figure 2.3), shown by linear and quadratic terms in the simple (quadratic) regression and in multiple regression (Table 2.1). When examined by stand origin, effect size was not affected by stand age in relatively young (3 – 35 years) planted forests, but decreased with stand age in relatively old (35 – 180 years) natural forests (Figure 2.3). Effect size increased with increasing experiment age in grasslands (Figure 2.3). The estimated relationship between effect size, species richness, experiment and stand age were similar between simple and multiple regressions (Table 2.1)

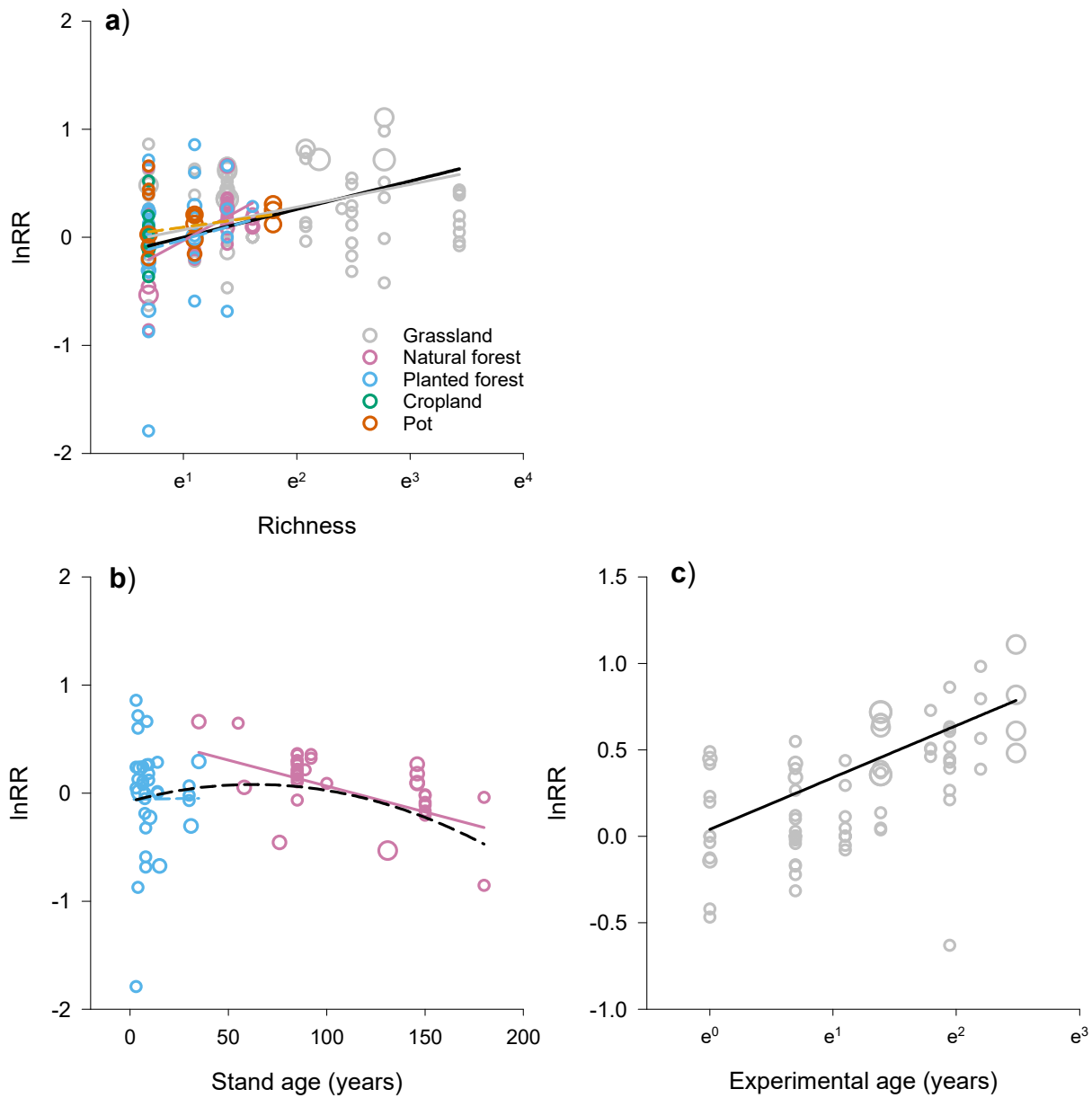


Figure 2.3. Determinants of diversity effects on fine root biomass. Partial regression plots show the natural log response ratios ($\ln RR$) of fine root biomass in mixtures to that in monocultures in relation to (a) richness, (b) stand age, and (c) experiment age from weighted bootstrapping multiple linear regression models (Table 2.1) once all other variables in the model are statistically controlled for. Black lines represent overall response across all ecosystem types (Table 2.1). Colors indicate ecosystem types: reddish purple—natural forest ($n = 35$), sky blue—planted forest ($n = 34$), grey—grassland ($n = 76$), bluish green—cropland ($n = 10$), and vermilion—pot ($n = 15$). The sizes of circles represent the relative weights of corresponding observations. Solid lines represent significant relationships ($P < 0.05$), and dashed lines insignificant ($P \geq 0.05$).

Table 2.1. Natural log-transformed response ratio ($\ln RR$) in relation to predictors determined by weighted (w_{rn}) multiple regression. Coefficients were bootstrapped with 4,999 iterations. Bivariate relationships are represented by the coefficients of simple or quadratic regression. Variables listed in boldface indicate significant effects ($P < 0.05$). The number in parentheses represents bootstrapped 95% confidence interval. $\ln(S)$, $\ln(\text{Exp age})$, R^2 , and P are natural log transformed species richness, natural log transformed experiment age, explained variance by the model, and significance of the model, respectively. Cropland was not included as there is no variation in species richness in mixtures.

| Ecosystem | Regression | Numbers of studies, observations | $\ln(S)$ | Stand age ($\times 10^{-2}$ years) | Stand age ² ($\times 10^{-4}$) | $\ln(\text{Exp age})$ | R^2 | P |
|-----------|------------------|----------------------------------|--------------------------|-------------------------------------|---|--------------------------|-------|--------|
| Overall | Simple | 48, 170 | 0.26 (0.15, 0.37) | | | | 0.34 | <0.001 |
| Forest | | | | | | | | |
| | Pooled Multiple | 26, 69 | 0.52 (0.21, 0.83) | 0.22 (-0.48, 0.92) | -0.5 (-1.0, 0) | | 0.22 | <0.001 |
| | Simple | | 0.51 (0.24, 0.78) | 0.44 (-0.26, 1.14) | -0.5 (-1.0, 0) | | | |
| | Natural Multiple | 14, 35 | 0.56 (0.23, 0.89) | -0.50 (-0.80, -0.20) | | | 0.58 | <0.001 |
| | Simple | | 0.60 (0.18, 1.02) | -0.48 (-0.87, -0.09) | | | | |
| | Planted Multiple | 12, 34 | 0.26 (-0.34, 0.86) | 0.03 (-0.10, 0.16) | | | 0.04 | 0.55 |
| | Simple | | 0.24 (-0.33, 0.81) | 0.05 (-1.36, 1.46) | | | | |
| Grassland | Multiple | 9, 76 | 0.19 (0.11, 0.27) | | | 0.29 (0.20, 0.38) | 0.60 | <0.001 |
| | Simple | | 0.22 (0.07, 0.37) | | | 0.31 (0.18, 0.44) | | |
| Pot | Simple | 9, 15 | 0.11 (-0.09, 0.31) | | | | 0.05 | 0.21 |

2.5 Discussion

Our results demonstrate a higher fine root biomass and annual production in mixtures compared with monocultures. This finding concurs with those for above-ground productivity (Cardinale et al. 2007, Zhang et al. 2012). Since fine root biomass was found to be strongly and linearly related to fine root biomass production, therefore, fine root biomass was used as a proxy for fine root biomass production in our study. Together, these results strongly suggest that mixtures have an overall positive effect on fine root productivity. Our results also show different effect sizes among ecosystem types. Although differences in abiotic environments among ecosystem types might have affected diversity effects below-ground (Mommer et al. 2010, Smith et al. 2013), our analysis indicated that the higher overyielding in grasslands was at least partly attributable to its higher richness in species mixtures (Appendix 4), as there was no difference in species richness's effect among ecosystem types (Appendix 5). The transgressive overyielding in grasslands indicating a strong complementary effects which could be another reason for its higher overyielding for fine root productivity. We postulate that the lack of diversity effects in young planted forests resulted from the fact that planted forests with initially low densities failed to fully occupy sites, and thus diversity effects may not have been expressed due to little inter-specific interactions with low densities or site occupancy (de Kroon et al. 2012).

Similar to those found for above-ground biomass (Cardinale et al. 2007, Zhang et al. 2012), the response ratio of the fine root biomass in mixtures to those in monocultures increased logarithmically with species richness. Moreover, the positive effects of species richness were similar across all ecosystem types. This positive relationship between fine root productivity and species richness observed in this study probably resulted from multiple

mechanisms associated with interspecific interactions including the improved resource partitioning, interspecific facilitation, and/or reduced pathogenic pressures in more species diverse communities, especially for grassland where transgressive overyielding was found. These finding extends our understanding of the important role of species richness in above-ground (Tilman et al. 2001) to below-ground, suggesting that species richness may serve as a reliable predictor for biomass production of the whole community.

Our results reveal a temporal change of diversity effect in grasslands. As we hypothesized, the response ratio of the fine root biomass in mixtures to those in monocultures increased with experiment age in grasslands. This finding agrees with Ravenek et al. (2014) who found that positive species richness effects on root biomass become apparent only four years after the establishment of the experiment. This temporal change may be attributable to the increased magnitude of niche complementarity over time. Together with previous studies of temporal changes of diversity effects for above-ground productivity in grasslands (Tilman et al. 2001, Cardinale et al. 2007), our finding further suggests that diversity effects on community productivity increase overtime in planted grasslands.

Temporal shifts of diversity effects on fine root productivity were also found in forests. However, unlike grassland, effect size responded quadratically to stand age in forests with a larger effect size in the intermediately aged than young or old stands. This pattern of age effects may reflect the data availability. The initial weak increase in age effects may represent the response of effect size in young forest plantations, whereas the diversity effects in natural forests decreased with stand age. The weak response of effect size to stand age in young forest plantations may be attributed to the fact that it usually takes multiple decades to centuries in forests for trees to reach maturation in high latitudes, and only then site resources

can be optimally used and the expression of species complementarity may be found (Cavard et al. 2011b, Brassard et al. 2013). The decrease phase of diversity effects on fine root productivity in natural forests contrast with what was found in aboveground (Zhang et al. 2012), suggesting that changes in biomass allocation between above- and below-ground with forest stand development may play a role. The reduced expression of diversity effects along stand age may also due to the abundant canopy gaps in old forests (Chen and Popadiouk 2002b). The different temporal trends found between forest communities and grassland communities likely reflect different temporal scales, i.e., a few years to one or two decades in grassland experimental studies and multiple decades to centuries in forest studies, as well as different origins in forest studies. Future studies are needed to further examine long-term responses in both experimental and natural systems.

In conclusion, our analysis shows that mixtures enhance fine root production and biomass. Our finding extends the understanding of diversity effects on above-ground biomass production (Cardinale et al. 2007, Zhang et al. 2012) and indicate the pattern of ‘overyielding’ could scale up to entire ecosystems. Additionally, our analysis demonstrates that the response ratio of the fine root biomass in mixtures to those in monocultures increase consistently with species richness across all terrestrial ecosystem types examined and also shift temporally in both grasslands and forests. Our results have relevance in calculating the total ecosystem production and carbon storage of terrestrial ecosystems, and can provide a broad guide for management practices aimed at increasing below-ground productivity and carbon sequestration. Our analysis call for future experiments to incorporate a wide range of stand ages in both natural and experimental systems to explore the potential mechanism for the temporal changes of diversity effects on community productivity.

CHAPTER 3: EFFECTS OF SPECIES DIVERSITY ON FINE ROOT PRODUCTIVITY INCREASE WITH STAND DEVELOPMENT AND ASSOCIATED MECHANISMS IN A BOREAL FOREST

3.1 Abstract

There is a growing interest in understanding the relationship between diversity and below-ground productivity due to the critical contribution of below-ground systems to overall terrestrial productivity. Yet, the temporal (seasonal and developmental) changes of diversity effects on below-ground productivity and their underlying mechanisms remain unclear. We hypothesized that: (i) diversity effects on fine root productivity increase with stand development, and (ii) increased diversity effects associated with stand development result from augmented horizontal soil space utilization, increased forest floor depth for rooting, enhanced effects in nutrients-poor soil layers, and/or foraging, toward high nutrient availability. We investigated the effects of tree species diversity on fine root productivity by sampling 18 stands dominated by single species and their mixtures in post-fire boreal forests of two stand ages (8 and 34 years following stand replacing fire). Species evenness was significantly higher in species mixtures than single species dominated stands at both age classes, while species richness did not differ across stand types and age classes. We found that the annual fine root production was higher in mixtures than the mean of single species dominated stands in both stand ages, with a significantly higher magnitude of effects in the 34-year-old than 8-year-old stands. Mixtures had higher horizontal soil volume filling than single species dominated stands with a more pronounced increase in the 34-year-old than 8-year-old stands. Compared with the 8-year-old stands, the 34-year-old stands had increased

forest floor depth and greater overyielding with soil depth, and their fine root productivity was more responsive to the vertical variation in soil phosphorus concentrations among soil layers. Our results provide evidence for increasing positive diversity effects on fine root productivity with stand development in heterogeneous natural forests. Moreover, our results indicate that the increased positive diversity effects with stand development was the result of multiple mechanisms, including higher horizontal soil volume filling, a thicker forest floor layer for rooting, a higher magnitude of complementarity in nutrient-poor deep soil layers, and stronger nutrient foraging toward soil layers with high nutrient concentrations in older than younger stands.

3.2 Introduction

The relationships between diversity and productivity (DPR) below-ground have recently attracted increasing attention because below-ground ecosystem processes make a critical contribution to overall terrestrial productivity (de Kroon et al. 2012). Previous empirical below-ground DPR studies in forest ecosystems, based on snapshots of a single stand development stage, or one stand age, have reported positive (Brassard et al. 2011b, Lei et al. 2012a, Brassard et al. 2013, Laclau et al. 2013, Lang'at et al. 2013) or insignificant (Bauhus et al. 2000, Meinen et al. 2009c, Jacob et al. 2013, Domisch et al. 2015) diversity effects on fine root productivity. These divergent findings may arise due to temporal variation in diversity effects associated with stand development (Zhang et al. 2012, Turnbull et al. 2013, Ma and Chen 2016). Additionally, when using fine root biomass to represent productivity, contrasting findings might occur due to seasonal variation associated with different sampling dates, as positive diversity effects on fine root biomass may occur only during sampling dates when demands for water and nutrients are high (Brassard et al. 2013). The lack of understanding of

potential temporal changes of diversity effects hampers a full appreciation of diversity effects on fine root productivity in forest ecosystems, in which productivity is strongly dependent on stand age or developmental stage (Yuan and Chen 2012).

Temporal changes of diversity effects on productivity were first revealed by Tilman et al. (2001) in a long-term grassland DPR experiment, showing that the impact of species richness on above-ground and total biomass became progressively stronger over time. In a meta-analysis, Cardinale et al. (2007) attributed this temporal change of diversity effects to the increased magnitude of complementarity over time. Reich et al. (2012) offered an alternative explanation, which posited that the functional redundancy of early years fades over time. For below-ground, positive species richness effects on root biomass were found to become apparent only four years following the establishment of the experiment (Ravenek et al. 2014). There is a lack of evidence of temporal changes of below-ground DPR in forests (Ma and Chen 2016), but, given the strong link between above- and below-ground in terrestrial ecosystems (Wardle 2002), diversity effects on below-ground productivity are expected to increase with stand development, similar to those in the above-ground (Zhang et al. 2012). More importantly, the underlying mechanisms for potential temporal changes of below-ground DPR remain unknown, particularly for long-lived forests, in which the processes associated with species complementarity might differ from those in experimental grasslands (Forrester and Bauhus 2016). Moreover, compared with experimental BEF studies in grasslands where species diversity is mostly represented by species richness due to little variation in species evenness, species evenness effects on productivity are highly pronounced in natural systems (Hillebrand et al. 2008, Zhang et al. 2012, Brassard et al. 2013).

Several mechanisms may be associated with increasing diversity effects on fine root productivity with stand development in forests. First, in mature stands, high productivity and biomass demand elevated water and nutrient uptake from fine roots, leading to intense root interactions (de Kroon 2007, de Kroon et al. 2012) and more evenly distributed roots horizontally to increase resource foraging in mixtures than single species dominated stands (Brassard et al. 2011b, Brassard et al. 2013) (Figure 3.1). By contrast, young stands with low root biomass (Yuan and Chen 2012) may under-utilize soil space and other resources (water and nutrients) with little resource competition and interspecific root interactions in species mixtures (de Kroon et al. 2012). Minimal interactions among roots may prevent the expression of diversity effects through resource partitioning and/or reduced competition (Hooper et al. 2005, Domisch et al. 2014, Forrester and Bauhus 2016), leading to a negligible diversity effect on root productivity and the alteration of horizontal fine root distribution (Figure 3.1). Second, increased horizontal filling in mature stands might occur simultaneously with the increased utilization of soil resources, and more robust fine roots overyielding in deeper soil layers (Brassard et al. 2013) (Figure 3.1). Third, low diversity effects in young fire-originating boreal forest stands may be attributed to the minimal forest floor depth that is available for roots, due to the reduction of the layer by stand-replacing fire (Shrestha and Chen 2010), i.e., reduced biotope space (soil volume) available for roots (Dimitrakopoulos and Schmid 2004) (Figure 3.1). Lastly, positive diversity effects on fine root productivity have been attributable to increased demands for foraging limited nutrients by distributing roots to vertical nutrient-rich layers (Mommer et al. 2010, Brassard et al. 2013, Ravenek et al. 2014). Due to their lesser demand for nutrients, roots in young stands may be less responsive to soil nutrients than those in mature stands.

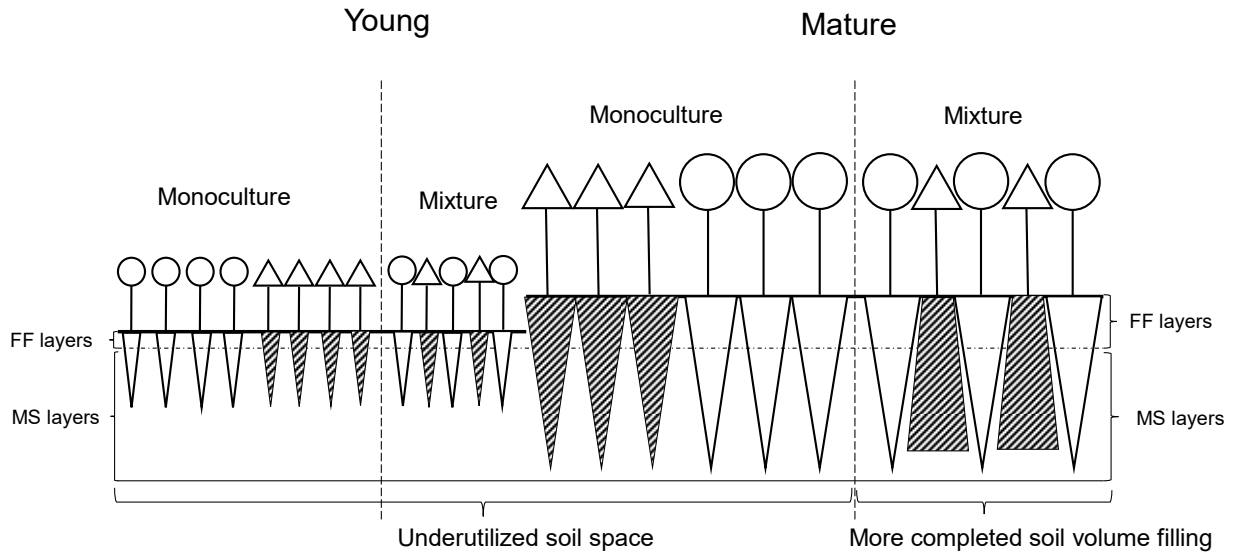


Figure 3.1. Potential mechanisms for temporal change of diversity effects on fine root productivity in forest ecosystems. FF and MS are forest floor and mineral soil, respectively.

Here, we examined the temporal pattern of diversity effects on fine root productivity, measured as annual fine root production and fine root biomass, in 8- and 34-year-old single species dominated and mixed stands of natural boreal forests. We specifically tested whether 1) diversity effects on fine root productivity increase with stand development, and 2) the increased diversity effects result from augmented horizontal soil space utilization, increased forest floor depth for rooting, enhanced overyielding in nutrients-poor soil layers, and/or foraging toward high nutrient availability.

3.3 Materials and methods

3.3.1 Study area

This study was conducted in the boreal forest located north of Lake Superior and west of Lake Nipigon, in the Upper English River Forest Region approximately 150 km north of Thunder Bay, Ontario, between 49°27' N to 49°38' N, and 89°29' W to 89°54' W. This region

is characterized by a moderately dry, cool climate with short summers. Mean annual temperature and mean annual precipitation from 1981 to 2010 were 1.9 °C and 824 mm, respectively, at the closest climatic station of Cameron Falls (Environment Canada 2016). The topographical features of this region were shaped by the retreat of the Laurentide Ice Sheet approximately ten millennia ago. The soils on the upland sites are relatively deep glacial tills of the Brunisolic order (Soil Classification Working Group 1998). The most common natural disturbance in the study area is predominately stand-replacing crown fire with an average fire return interval of approximately 100 years over the last century (Senici et al. 2010).

3.3.2 Sampling design

At mesic sites in the study area, which support a wide range of forest compositions due to the variation of local propagule availability (Ilisson and Chen 2009), we sampled two post-fire stand age classes (i.e., 8 and 34 years since fire) and three overstory types (single-species stands dominated by *Populus tremuloides* Michx. (*Populus*) and dominated by *Pinus banksiana* Lamb. (*Pinus*) to mixtures (*Populus+Pinus*)). Each of the stand age classes and overstory types was replicated three times. Stand ages were derived from fire records and verified by sampling dominant trees (Senici et al. 2010). Similar to other studies that investigated tree species diversity effects in naturally established stands (Brassard et al. 2011b, Brassard et al. 2013), and following the definitions for single- and mixed-species stands in the forest resource inventory, the criteria for stand selection were that single-species-dominated stands contained a $\geq 80\%$ stand basal area of a single species, while in mixed-species stands none of the component species had a $\geq 80\%$ stand basal area.

All selected stands were naturally established following stand-replacing crown fires, and developed without silvicultural treatments. Sample stands were allocated several kilometers apart from each other, by selecting them from different road accesses to minimize neighbourhood influences and unknown environmental influences that might be spatially correlated. To assist in ensuring that tree species composition comprised the only significant source of variation among the stands, all stands were selected on mesic sites using an ecological classification approach (Taylor 2000), by allocating all sites on mid-slope positions of well-drained glacial moraines with >50 cm in thickness. The soil moisture regime class was confirmed by a soil profile examination, dug to the parent material, within each selected stand. The similarity of the sites was further validated through a comparison of the physical and chemical properties of soils; that is, the concentrations of total nitrogen and total carbon, cation exchange capacity, and soil texture composition of the mineral soil at a depth of 30-55 cm, following the method described by Laganriere et al. (2012).

3.3.3 Data collection

For each sample stand, a circular plot (400 m² in the 34-year-old stands, and 50 m² in the 8-year-old stands due to high stem densities) (Hart and Chen 2008) was randomly established to represent the stand. The diameter at breast-height (DBH), taken at 1.3 m above the root collar, height and species of all live trees with DBH ≥ 2 cm were measured and recorded. Stand basal areas by species were summed to the plot level and used for assigning stand-type classification (Table 3.1). Similar to the natural stands studied by Brassard et al. (2013), there was higher species evenness in species mixtures than single species dominated stands, but no difference in species richness (Table 3.1). Across stand ages and types, species evenness had little correlation to species richness ($r = 0.10$, $P = 0.153$).

Within each plot, seven soil cores (6.6 cm diameter) were randomly extracted from the forest floor to a mineral soil depth of 30 cm using a power auger, every month during the 2014 growing season (May to October), and May 2015. In northern forests, where frozen and snow-packed soils hinder sampling, fine root production during the winter months was assumed to be negligible (Steele et al. 1997, Konopka et al. 2005). To facilitate extraction by layer, and to minimize compaction during coring, we extracted the forest floor layer (the depth of the forest floor was then recorded, see Table 3.1), and subsequently, two mineral soil sections: MS1 (0-15 cm) and MS2 (15-30 cm), following the removal of the upper layer. In total, we extracted 756 cores for roots, and 180 cores for soil (each separated into three layers), resulting in 2808 samples for laboratory analysis.

Soil cores were transported in an ice-filled cooler from the field to the laboratory and stored in a freezer at -18°C until they were processed. These samples were initially soaked in water to separate roots from the soil, and then hand sorted to remove visible roots and coarse fragments. The remaining material was further gently washed over a sieve (0.5 mm mesh size) to remove the remaining root fragments from the soil. Fine roots (< Ø2 mm, determined using calipers) were selected and further sorted according to their status (live versus dead). Live roots were pale-colored on the exterior, elastic and flexible, free of decay, and had a whitish cortex, whereas dead roots were brown or black, rigid, and inflexible (broke easily), were in various stages of decay, and had a darker cortex (Persson 1983). The 'live' and 'dead' root components were then separately oven-dried to a constant mass at 65 °C and weighed.

Soil nutrients for all sites in this study have been previously reported (Hume et al. 2016). In brief, soil samples were collected from ten random points within each plot, separated by layers, and transported to the laboratory for chemical analysis. Soil samples

Table 3.1. Characteristics (mean and 1 s.e.m., $n = 3$) of the study stands in Northwestern Ontario, Canada. Stand types are single-species *Pinus banksiana* dominated (*Pinus*), single-species *Populus tremuloides* dominated (*Populus*), and their mixtures (*Populus+Pinus*).

| Stand type | 8-years post fire | | | 34-years post fire | | |
|---|-------------------|----------------|----------------------|--------------------|----------------|----------------------|
| | <i>Pinus</i> | <i>Populus</i> | <i>Populus+Pinus</i> | <i>Pinus</i> | <i>Populus</i> | <i>Populus+Pinus</i> |
| Stand basal area (m ² ha ⁻¹) | 1.55 ± 0.35b | 0.93 ± 0.33b | 1.39 ± 0.24b | 21.54 ± 1.07a | 25.56 ± 0.7a | 22.98 ± 1.57a |
| Stand density (trees ha ⁻¹) | 5933 ± 1790a | 11600 ± 4148a | 9200 ± 1301a | 4516 ± 601b | 3533 ± 92b | 3358 ± 140b |
| Tree species richness | 2.67 ± 0.33a | 2.67 ± 0.33a | 4.33 ± 0.33a | 2.00 ± 0.58a | 3.33 ± 0.88a | 2.67 ± 0.33a |
| Tree species evenness | 0.13 ± 0.04bc | 0.29 ± 0.07b | 0.57 ± 0.04ab | 0.12 ± 0.11bc | 0.23 ± 0.09b | 0.82 ± 0.11a |
| Tree species composition (% of stand basal area) | | | | | | |
| <i>Pinus banksiana</i> | 97.94 ± 1.03 | 2.96 ± 2.95 | 67 ± 2.31 | 96.31 ± 3.34 | 1.37 ± 0.87 | 40.92 ± 8.74 |
| <i>Populus tremuloides</i> | 1.21 ± 1.11 | 91.86 ± 3.08 | 25.13 ± 2.08 | 3.08 ± 2.73 | 92.5 ± 3.54 | 52.12 ± 6.48 |
| <i>Picea mariana</i> | 0 | 0 | 0 | 0 | 0 | 6.95 ± 6.05 |
| <i>Salix</i> spp. | 0.54 ± 0.27 | 1.77 ± 0.94 | 0.57 ± 0.41 | 0 | 1.69 ± 1.38 | 0 |
| <i>Picea glauca</i> | 0 | 0 | 0 | 0 | 4.12 ± 3.64 | 0 |
| <i>Prunus pensylvanica</i> | 0 | 0.41 ± 0.41 | 1.03 ± 0.72 | 0 | 0 | 0 |
| <i>Betula papyrifera</i> | 0.63 ± 0.63 | 3.01 ± 3.01 | 6.27 ± 3.95 | 0.61 ± 0.61 | 0 | 0 |
| Forest floor depth | 2.76 ± 0.17b | 1.89 ± 0.75b | 1.52 ± 0.35b | 7.34 ± 0.58a | 6.33 ± 0.31a | 6.76 ± 0.41a |

Differences in characteristics among stand types and ages were tested using a two-way ANOVA. Different letters indicate a significant difference ($\alpha = 0.05$).

were air-dried and stones were removed using a 2 mm sieve. Chemical analysis was conducted with samples ground finely to pass through a 100-mesh (0.15 mm) sieve to ensure the uniformity of the samples. Total N concentrations were analyzed via the dynamic flash combustion method, using a high temperature reactor to fully combust each sample, whereas gas chromatographic separation and thermal conductivity detection systems were employed to provide a precise measure of the quantity of elemental gases per 2 g sample (Carter and Gregorich 2008). We utilized the nitric/hydrochloric acid digestion method and inductively coupled plasma atomic emission spectrometry (ICP-AES) to determine the total P (Kalra and Maynard 1991). Nutrient concentrations were expressed as g kg^{-1} .

3.3.4 Statistical analysis

Fine root biomass (live roots) and necromass (dead roots) (Mg ha^{-1}) were calculated for each sampling date at each site by summing the dry weight of live and dead fine roots in each soil core and scaled up to per ha. Estimates of annual fine root production ($\text{Mg ha}^{-1} \text{ year}^{-1}$) were calculated by determining changes in dry weights for all sampling dates using a simplified decision matrix method (Yuan and Chen 2013). To quantify horizontal soil volume utilization, we calculated a horizontal homogeneity index as the reciprocal of standard deviation of the biomass values of all soil layers, combined among the seven soil cores for each sampling date within each sample plot. A higher homogeneity index value would imply a more homogenous horizontal distribution of biomass among the soil cores (Brassard et al. 2013). To test whether roots may forage for nutrients vertically to nutrient-rich layers, we quantified fine root production per cm of soil depth for each soil layer.

The effects of species mixtures on productivity were calculated as the ratio (R_o) of the observed productivity value ($P_{observed}$) to the expected value ($P_{expected}$, the weighted average

monoculture yield of the component species) of fine root annual production or biomass in mixtures (Loreau and Hector 2001).

$$R_o = \frac{P_{observed}}{P_{expected}} \quad (1)$$

$$P_{expected} = \sum (P_i \times m_i) \quad (2)$$

where P_i is the observed fine root production or biomass of species i in pure stands, and m_i is the proportion of stand basal area of species i in the mixture. If the 95% confidence intervals of the R_o do not cover one, species mixture has a significant (positive or negative) impact on fine roots annual production or biomass. Transgressive overyielding was also tested by the ratio (R_t) of the observed production or biomass in mixtures ($P_{observed}$) to that of the observed highest yielding monocultures of the component species ($P_{highest}$).

$$R_t = \frac{P_{observed}}{P_{highest}} \quad (3)$$

For our first hypothesis (i.e., diversity effects increase with stand development), we tested the effect of stand age on R_o and R_t for annual production using one-way analysis of variance (ANOVA). The effects of stand age and sampling date on R_o and R_t for fine root biomass were tested using repeated measure ANOVA since the sampling date reflected a repeated measure of each sample stand. Additionally, we tested whether annual fine root production differed among stand types and stand age using two-way ANOVA. We used a repeated measure ANOVA to examine the effects of stand type, age and sampling date on fine root biomass.

For our second hypothesis, we quantified R_o and R_t (eqns. 1-3) as measures of diversity effects on homogeneity index. We first tested whether the R_o and R_t of the horizontal homogeneity index of fine root biomass increased with stand age using a repeated measure

ANOVA. We also tested whether the horizontal homogeneity index differed among stand types, stand age, and sampling date. The relationship between fine root biomass and homogeneity index was tested using type II regression, which is a method recommended when variables cannot be clearly distinguished as independent or dependent (Legendre 2015). Second, we tested whether the forest floor depth increased with stand age. Third, we investigated whether the R_o and R_t of annual fine production increased with soil depth. Lastly, we tested whether the relationships between fine root production and soil N and P concentrations differed with stand age using analysis of covariance. Assumptions of normality and homogeneous variance were examined by Shapiro-Wilk's test and Leven's test, respectively. Assumption of sphericity for repeated measure ANOVA was verified by Mauchy's test. While the assumptions of normality and sphericity were met for all analyses, the assumption of homogeneity were not for some analyses. Natural logarithm transformation on the respective dependent variable was used, and the assumption was met following the transformation. All statistical analyses were conducted with R (version 3.1.2).

3.4 Results

The effect of species diversity on annual fine root production was marginally higher in the 34- than the 8-year-old stands (Table 3.2, $P=0.119$), showing 32% and 74% higher production in the mixtures than the mean of single species dominated stands in the 8- and 34-year-old stands, respectively (Figure 3.2). Transgressive overyielding occurred in the 34-year-old stands, but not in the 8-year-old stands (Figure 3.2). Annual fine root production differed among stand types with a marginally significant interaction effect of stand type and stand age (Table 3.3). Annual production was higher in the 8- year-old, rather than the 34-year-old

Table 3.2. Effect of stand age on mixture effect. Mixture effects represent by R_o (eqn. 1) and R_t (eqn. 3)

| Source | d.f. | R_o | | R_t | |
|-----------|------|-------|-------|-------|-------|
| | | MS | P | MS | P |
| Stand age | 1 | 0.27 | 0.119 | 0.79 | 0.005 |
| Error | 4 | 0.07 | | 0.03 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (P).

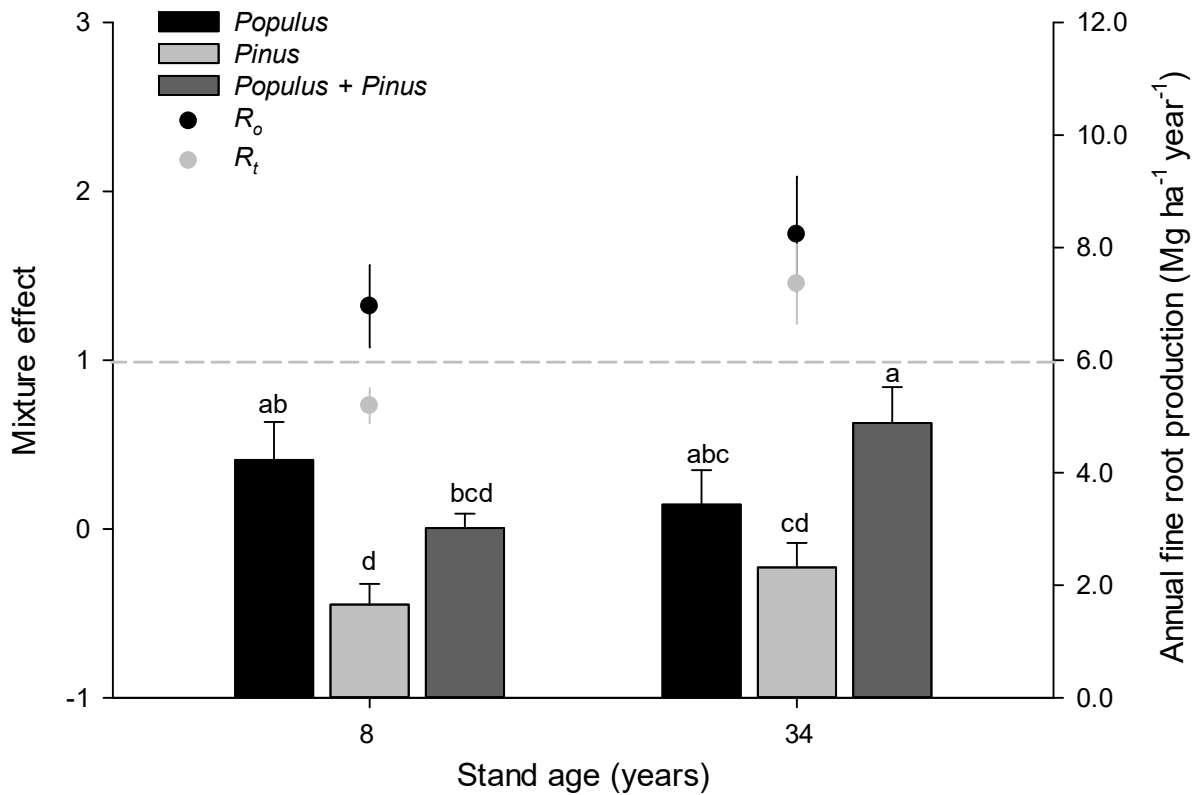


Figure 3.2. Effects of mixture on annual fine root production in relation to stand development. Mean and 95% confidence intervals of R_o and R_t higher than 1 indicate overyielding and transgressive overyielding of annual fine root production, respectively. Stand types were single-species *Populus tremuloides* dominated (*Populus*), single-species *Pinus banksiana* dominated (*Pinus*), and their even mixture (*Populus + Pinus*). Error bars represent 1 s.e.m. ($n = 3$).

Table 3.3. Effects of stand type and age on annual fine root production.

| Source | d.f. | Annual production | |
|------------------------|------|-------------------|----------|
| | | MS | <i>P</i> |
| Stand type | 2 | 7.26 | 0.004 |
| Stand age | 1 | 1.50 | 0.199 |
| Stand type × Stand age | 2 | 2.67 | 0.073 |
| Error | 12 | 0.81 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (*P*).

Populus stands, however, it was higher in the 34- year-old than the 8-year-old *Pinus* stands and mixtures (Figure 3.2).

For fine root biomass, both R_o and R_i differed significantly with sampling date, and significant overyielding of fine root biomass occurred only in the summer months for both stand ages, with a greater magnitude for 34-year-old than 8- year-old stands (Table 3.4, Figure 3.3). Transgressive overyielding occurred only during the summer in the 34-year-old stands (Figure 3.3b). For all stand types for both ages, fine root biomass peaked in the summer months (July to September) (Figure 3.3a and b, Table 3.5).

The effect of species diversity on the horizontal homogeneity index of fine root biomass was marginally higher in the 34-year-old than 8-year-old stands (Table 3.6, $P = 0.113$). The horizontal homogeneity index of fine root biomass was greater in mixtures than the mean of single species dominated stands, particularly in the 34-year-old stands (Figure 3.3c and d). Like fine root biomass, the homogeneity index peaked in the summer months (July to September) for all stand types and ages (Figure 3.4). The homogeneity index differed with stand type and sampling date, with a marginally significant interaction of stand age and

stand type (Table 3.5, $P=0.081$). Across stand type, ages and sampling date, the horizontal homogeneity index was positively associated with fine root biomass (Figure 3.5).

Table 3.4. Effects of stand age and sampling date on fine root biomass overyielding (R_o , eqn. 1) and transgressive overyielding (R_t , eqn. 3).

| Source | d.f. | R_o | | R_t | |
|----------------------------------|------|-------|--------|-------|-------|
| | | MS | P | MS | P |
| <i>Between subject</i> | | | | | |
| Stand age | 1 | 0.030 | 0.269 | 0.082 | 0.081 |
| Error | 4 | 0.018 | | 0.015 | |
| <i>Within subject</i> | | | | | |
| Sampling date | 5 | 0.098 | <0.001 | 0.066 | 0.004 |
| Stand age \times Sampling date | 5 | 0.045 | 0.006 | 0.030 | 0.089 |
| Sampling error | 20 | 0.010 | | 0.014 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (P).

Table 3.5. Effects of stand type, age and sampling date on fine root biomass and homogeneity index.

| Source | d.f. | Biomass | | Homogeneity | |
|--|------|---------|--------|-------------|--------|
| | | MS | P | MS | P |
| <i>Between subject</i> | | | | | |
| Stand type | 2 | 46.50 | <0.001 | 0.304 | <0.001 |
| Stand age | 1 | 137.98 | <0.001 | 0.016 | 0.293 |
| Stand type \times Stand age | 2 | 16.63 | <0.001 | 0.042 | 0.081 |
| Error | 12 | 0.41 | | 0.013 | |
| <i>Within subject</i> | | | | | |
| Sampling date | 5 | 19.41 | <0.001 | 0.211 | <0.001 |
| Stand type \times Sampling date | 10 | 3.10 | <0.001 | 0.015 | 0.206 |
| Stand age \times Sampling date | 5 | 1.01 | 0.06 | 0.029 | 0.034 |
| Stand type \times Stand age \times Sampling date | 10 | 0.70 | 0.15 | 0.002 | 0.996 |
| Sampling error | 60 | 0.45 | | 0.011 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (P).

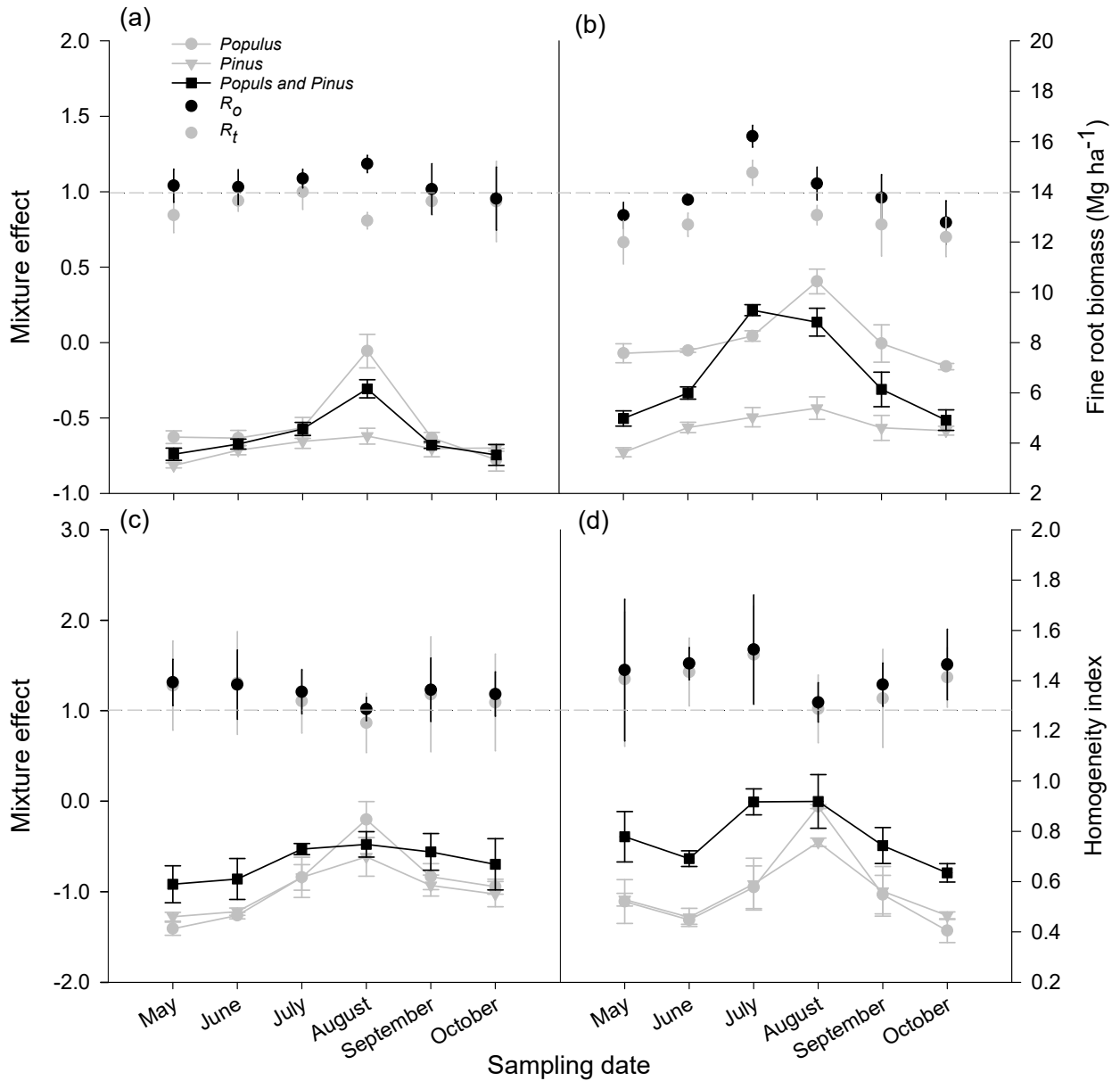


Figure 3.3. Effects of mixture on fine root biomass and homogeneity index along sampling date for each stand age. (a, c) 8-year-old stands, (b, d) 34-year-old stands. Mean and 95% confidence intervals of R_0 and R_t higher than 1 indicate overyielding and transgressive overyielding of fine root biomass or homogeneity index, respectively. Stand types are described in Figure 3.2. Error bars represent 1 s.e.m. ($n = 3$).

Table 3.6. Effects of stand age and sampling date on horizontal homogeneity index of fine root biomass (R_o , eqn. 1) and (R_t , eqn. 3).

| Source | d.f. | R_o | | R_t | |
|----------------------------------|------|-------|-------|-------|-------|
| | | MS | P | MS | P |
| Between subject | | | | | |
| Stand age | 1 | 0.422 | 0.113 | 0.303 | 0.172 |
| Error | 4 | 0.103 | | 0.109 | |
| Within subject | | | | | |
| Sampling date | 5 | 0.122 | 0.162 | 0.158 | 0.065 |
| Stand age \times Sampling date | 5 | 0.038 | 0.731 | 0.056 | 0.510 |
| Sampling error | 20 | 0.068 | | 0.063 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (P).

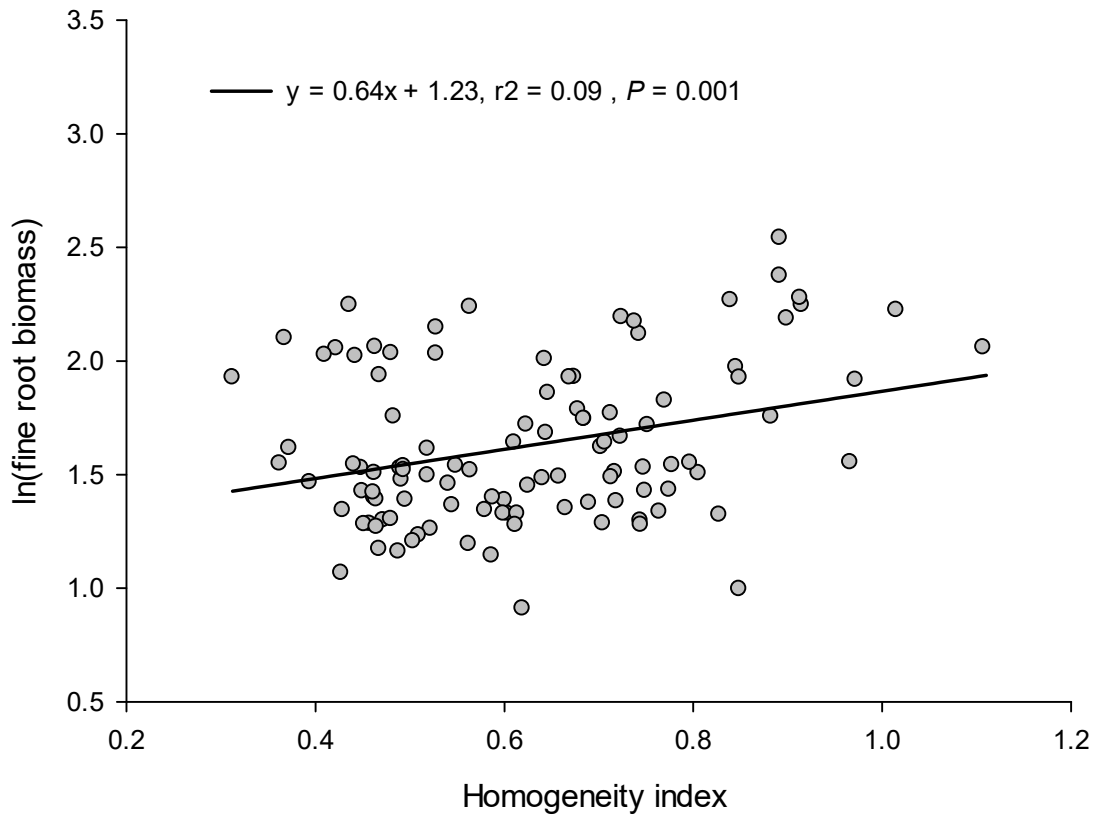


Figure 3.5. Relationship between natural log transformed fine root biomass and homogeneity index. Type II regression were used to fit the model.

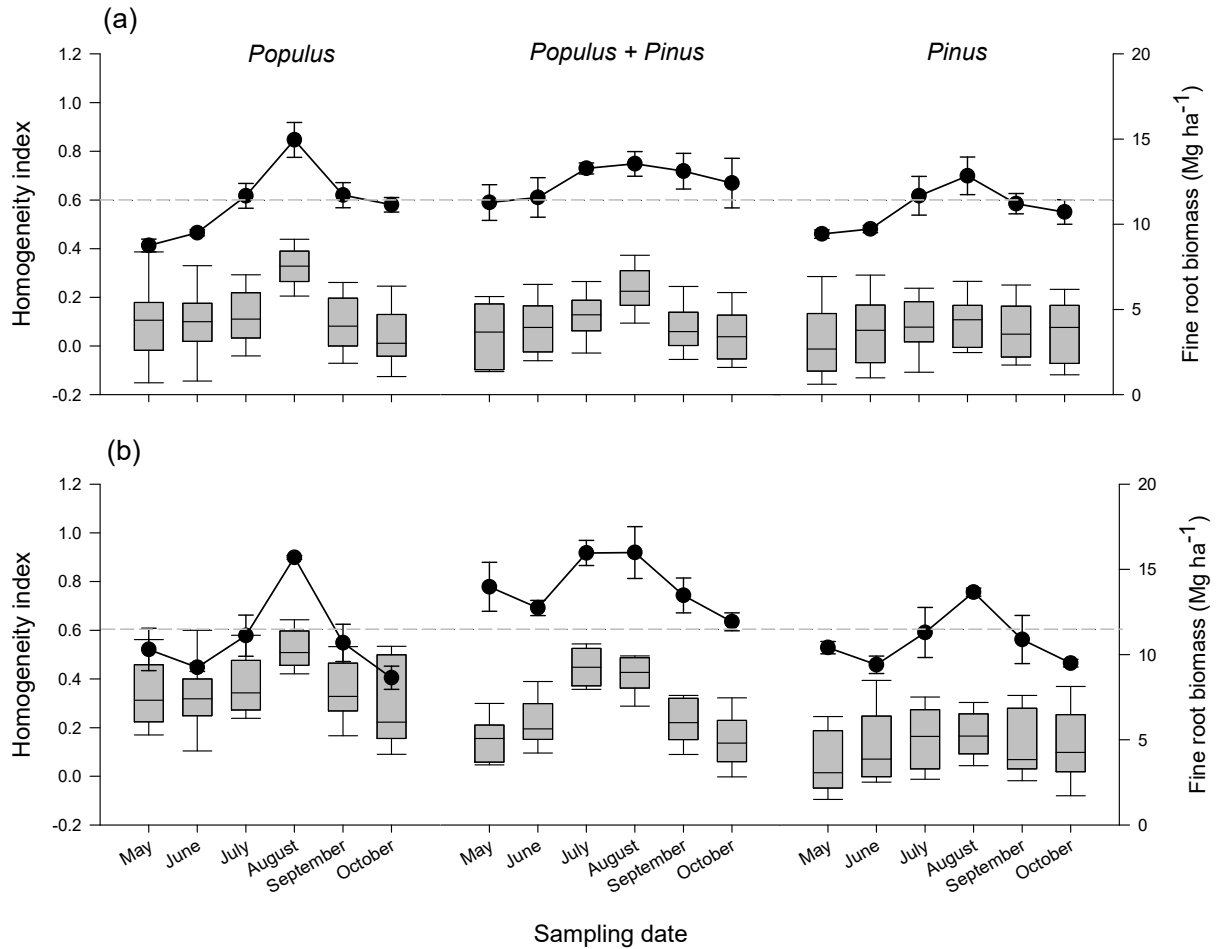


Figure 3.4. Spatial variation of fine root biomass (box plots) and corresponding horizontal homogeneity index (circles and lines) in relation to the sampling date for each stand type and age. Box plots show the median (line within the box), 25th and 75th percentiles (the boundaries of the box) and 90th and 10th percentiles (error bars) of mean fine root biomass of seven soil cores, which were ranked from large to small within each plot, of the three replicate plots. (a) 8-year-old stands, (b) 34-year-old stands. Error bars represent 1 s.e.m. ($n = 3$).

As the forest floor depth increased with stand development (Table 3.1), fine root production was allocated more to the forest floor layer in the 34- than 8-year-old stands (Figure 3.6a, b). Mixture effects did not differ significantly with soil depth in the 8-year-old stands, but increased significantly with soil depth in the 34-year-old stands (Figure 3.6a, b). Fine root production (per cm soil depth) increased with N concentration across both stand age

classes, and increased with P concentration in the 34-year-old, but not in the 8-year-old stands (Figure 3.6c-f, Table 3.7).

3.5 Discussion

We found that the effects of high species evenness in mixtures, dominated by two boreal tree species differing phylogenetically (coniferous vs. broad-leaved) (Cadotte et al. 2009, Valverde-Barrantes et al. 2015), on fine root productivity increased with stand age. In alignment with a previous study conducted in the same area (Brassard et al. 2013) that found a transgressive overyielding of fine root production in a mixture of trembling aspen and jack pine of 85-year-old stands, our results indicated that positive diversity effects on annual fine root production increase with stand development in natural boreal forests. Our results also revealed that diversity effects on fine root biomass occurred only during the summer months, with a greater magnitude in the older than younger stands. Importantly, the increased positive diversity effects on both annual fine root production and fine root biomass along the stand development observed in this study could have resulted from the increased strength of species complementarity, as transgressive overyielding of both annual fine root production and fine root biomass occurred in the older but not younger stands. This is consistent with previous findings that the strength of species complementarity increases over time for above-ground biomass and productivity in forests (Zhang et al. 2012, Lasky et al. 2014), below-ground biomass (Ravenek et al. 2014), and total biomass in grassland experiments (Tilman et al. 2001, Reich et al. 2012), indicating that diversity effects on above- and below-ground productivity occur in tandem through stand development.

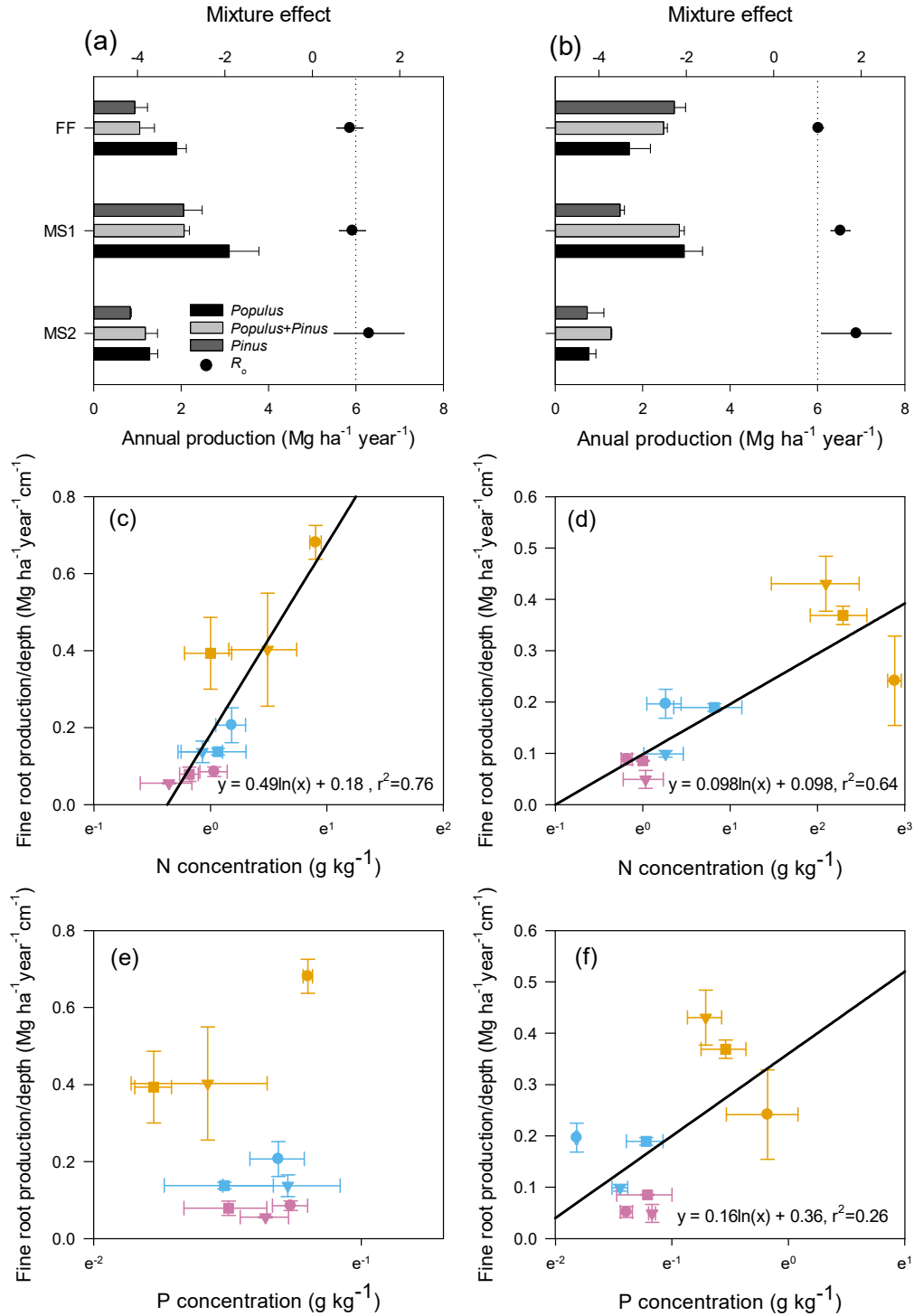


Figure 3.6. Annual fine root production in relation to soil layers. Annual fine root production by stand type (Error bars represent 1 s.e.m., $n = 3$) and mixture effects (calculated as overyielding, R_o mean and 95% confidence intervals) in 8-year-old and 34-year-old stands. Relationships between annual fine root production per soil depth and N concentrations (c, d) and P concentrations (e, f) in 8-year-old and 34-year-old stands, respectively. Colors indicate soil layers: vermilion—Forest floor, sky blue—MS1, and reddish purple—MS2. Shapes

indicate stand type: circle—Populus stands, triangle—Pinus stands, and square—Populus + Pinus stands. Fitted lines represent significant relationships ($P < 0.05$). Dots and error bars represent mean values and 1 s.e.m. ($n = 3$).

Table 3.7. Effects of stand age and type on the relationship between fine root production per soil depth and nutrient concentration. N and P means nitrogen and phosphorus concentration, respectively.

| Source | d.f. | MS | <i>P</i> |
|--|------|-------|----------|
| <i>ln</i> (N) | 1 | 0.286 | <0.001 |
| Stand age | 2 | 0.204 | 0.003 |
| Stand type | 2 | 0.001 | 0.963 |
| <i>ln</i> (N) × Stand age | 1 | 0.119 | 0.021 |
| <i>ln</i> (N) × Stand type | 2 | 0.009 | 0.627 |
| Stand age × Stand type | 2 | 0.006 | 0.752 |
| <i>ln</i> (N) × Stand age × Stand type | 2 | 0.162 | 0.001 |
| Error | 42 | 0.02 | |
| <i>ln</i> (P) | 1 | 0.014 | 0.498 |
| Stand age | 2 | 0.064 | 0.153 |
| Stand type | 2 | 0.009 | 0.747 |
| <i>ln</i> (P) × Stand age | 1 | 0.087 | 0.097 |
| <i>ln</i> (P) × Stand type | 2 | 0.055 | 0.176 |
| Stand age × Stand type | 2 | 0.049 | 0.207 |
| <i>ln</i> (P) × Stand age × Stand type | 2 | 0.082 | 0.077 |
| Error | 42 | 0.03 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (*P*).

Our results indicate that multiple associated mechanisms may be responsible for increased diversity effects on fine root productivity with stand development in natural forests. We found higher fine root horizontal homogeneity in mixtures than expected from their respective single-species-dominated stands, in both stand age classes. There was a stronger alteration of fine root horizontal distribution for the realized greater magnitude of diversity effects on fine root productivity in mature than young stands. These findings are consistent with our expectations that low root biomass and productivity in young stands (Yuan and Chen 2012) under-utilize soil spaces with little root interaction, hence there is a minimal alteration of horizontal fine root distribution (de Kroon 2007, de Kroon et al. 2012, Domisch et al. 2015).

As stands developed in our study forests, depth of nutrient-rich forest floor increased. Our findings of increased diversity effects on fine root productivity with stand development could also be attributed to increased soil volume for roots (Dimitrakopoulos and Schmid 2004). Moreover, we found that fine root overyielding increased with soil depth in the 34-year-old stands, suggesting that growing into deeper soil layers is an additional strategy employed to satisfy the requirement for more resources in the support of greater above- and below-ground productivity in species mixtures in mature stands (Brassard et al. 2013, Mueller et al. 2013). We also found that fine root production was positively associated with the N concentration of soil layers across all stand types and ages. This finding suggests that N foraging is ubiquitous regardless of stand type and age (Mommer et al. 2010, Brassard et al. 2013, Ravenek et al. 2014), and indicated that higher fine root productivity in species mixtures benefited in part from the N foraging behaviour. More importantly, we found a positive relationship between soil P concentration and fine root production, suggesting a

stronger demand for fine roots to forage for P, in the 34-year-old stands. This is likely because soil P is more limiting than soil N, indicated by a higher N to P ratio in the 34-year-old than in 8-year-old stands (Hume et al. 2016). We note that the positive relationship between soil P and fine root production in the 34-year-old stands was driven by high P concentration in the forest floor (Figure 3.6f). Moreover, both soil N and P were higher in the top mineral layer of mixtures than those of single species dominated stands in the 34-year-old stands, but not in the 8-year-old stands (Figure 3.6c-f), suggesting that positive diversity effects on soil fertility increase over time (Dybzinski et al. 2008). Furthermore, mycorrhizal diversity strongly influences plant biodiversity and ecosystem productivity (Sanders et al. 1998). *Pinus* and *Populus* tend to host different mycorrhizal compositions (Kernaghan et al. 2003). It remains to be examined whether the increased diversity effects on fine root productivity with stand development in our study forest could be attributed to increasing mycorrhizal diversity.

In summary, by studying the diversity effects on fine root biomass and production with stand development in a natural boreal forest, we provide evidence of increasing diversity effects on fine root productivity with stand development. Moreover, we found that the increased diversity effects with stand development might have resulted from multiple processes: increasing horizontal and vertical soil volume filling, increasing forest floor depth/volume, and foraging limiting soil nutrients all resulting in more complete use of soil space and nutrients, while benefiting from increasing soil nutrient inputs and retention. We note that these processes operated simultaneously. Our results offer a new and important understanding of the temporal dynamics of the relationship between biodiversity and ecosystem function.

CHAPTER 4: POSITIVE SPECIES MIXTURE EFFECTS ON FINE ROOT TURNOVER AND MORTALITY INCREASE WITH STAND DEVELOPMENT IN NATURAL BOREAL FORESTS

4.1 Abstract

The positive species mixture effects on both above- and below-ground productivity have been well documented in diverse ecosystem types. However, whether the results obtained on productivity can be generalized to other ecosystem processes remains patchy. We investigated the effects of tree species mixtures on fine root biomass turnover and mortality by sampling 18 stands dominated by single species and their mixtures in post-fire boreal forests of two stand ages (8 and 34 years following stand-replacing fire). Fine root biomass turnover and mortality were higher in mixtures than the mean of single-species-dominated stands in both stand ages, with higher mixture effects in the 34-years-old than in the 8-year-old stands. Mixture effects on turnover and mortality did not differ with soil depth in 8-year-old stands, but turnover increased while mortality decreased with increasing soil depth in 34-years-old stands. Both turnover and mortality significantly increased with tree species evenness in 34-year-old stands, but not in 8-year-old stands. Root turnover and mortality were positively associated with annual fine root production across all stand types and ages. Our results provide the first evidence for increasingly positive mixture effects on fine root biomass turnover and mortality with stand development in heterogeneous natural forests. Moreover, our results suggest that the increased mixture effects with stand development

resulted from increased competition intensity induced by the overyielding of fine root biomass production in mixtures.

4.2 Introduction

In recent decades, the biodiversity and ecosystem functioning relationship (BEF) has been a major ecological research focus to help understand the impact of global species extinction crisis on ecosystem functioning (Cardinale et al. 2012). Species mixtures positively affect both above- and belowground productivity in diverse ecosystem types (Cardinale et al. 2012, Zhang et al. 2012, Liang et al. 2016, Ma and Chen 2016, Duffy et al. 2017). However, whether the positive mixture effects on productivity can be generalized to other ecosystem processes remains patchy, especially for belowground processes in long-lived natural forests. In terrestrial ecosystems, fine roots ($\varnothing \leq 2\text{mm}$) with high turnover and mortality rates are a major contributor to nutrient cycling and carbon accumulation, translocating carbon and nutrients from roots to the long-lasting soil organic pool (Richter et al. 1999, Tefs and Gleixner 2012). In the boreal forest, approximately 50 to 70% of soil carbon results from the mortality of roots and root-associated microorganisms (Clemmensen et al. 2013). Unlike leaves, the timing and rate of root growth and root death are difficult to study, especially at the stand level. This is because sampling plant roots to represent the overall root pool of the stand is destructive, laborious and technically challenging (Hendricks et al. 2006, Brassard et al. 2009). Particularly, although fine root mortality and turnover differ with species traits and root sizes (Chen and Brassard 2013, McCormack et al. 2015, McCormack et al. 2017), detailed maps of fine root distributions are currently almost impossible to construct at the stand level (Brassard et al. 2011b). Hence this part of the belowground function continues to be one of the most challenging issues in BEF studies. The limited appreciation of the effects

of species diversity on fine root turnover and mortality is a major omission in our understanding of how diversity affects ecosystem functions and hinders efforts to model terrestrial biogeochemistry (Ostle et al. 2009).

A few existing studies on the diversity–fine root biomass turnover relationships, conducted in temperate forests at the stand level, have reported contrasting results. Fine root biomass turnover rate was found to increase with tree species richness in an 80-160 year-old natural temperate forest (Jacob et al. 2014) as well as in a 5-6 year-old temperate plantation (Lei et al. 2012a). In contrast, a study conducted in an 8-14 year-old temperate plantation did not observe higher biomass turnover rate in mixtures than monocultures (Domisch et al. 2015). Interestingly, all three studies have attributed high biomass turnover rates to high competition intensity in species mixtures. These attributions are plausible since competition as a stressor decreases fine root longevity due to the reduced soil resource availability associated with intensive competition, which makes fine roots progressively less efficient at obtaining soil resources because their maintenance costs exceed the benefits of resource acquisition (Chen and Brassard 2013, McCormack and Guo 2014). Niche theory suggests that intra-specific competition is stronger than interspecific competition (Loreau and Hector 2001, Cavard et al. 2011b). However, belowground competition is higher in mixtures than in monocultures due to their higher fine root production and biomass (Beyer et al. 2013, Brassard et al. 2013, Ma and Chen 2017). We first hypothesized that fine root biomass turnover and mortality would be higher in species mixtures than in monocultures since above- and belowground biomass and production are higher in species-rich than species-poor forests (Zhang et al. 2012, Ma and Chen 2016). This is because high fine root production and biomass lead to great resource scarcity, and reduce root lifespans and increase turnover rates

(Luke McCormack et al. 2012, Chen and Brassard 2013, McCormack et al. 2014, McCormack and Guo 2014). Secondly, since positive mixture effects on productivity tend to increase with stand development (Zhang et al. 2012, Ma and Chen 2017), we hypothesized that positive mixture effects on turnover mortality would increase with stand development.

Mixture effects on turnover rate may also change with soil depth. When stimulated by more resource competition such as higher production and biomass in species mixtures, plants can adjust their rooting depths and grow more fine roots to soil layers with more resources or with less root competition (Brassard et al. 2013, Mueller et al. 2013, Ma and Chen 2017). These rooting strategies may change with stand development. Low root biomass in young stands (Yuan and Chen 2012) may underutilize soil space and other resources (water and nutrients) with little resource competition and interspecific root interaction in mixtures (de Kroon et al. 2012), which may result in minimal alteration of fine root distribution (Ma and Chen 2017). Increasing root biomass and production with stand development (Yuan and Chen 2012), as well as increasing tree sizes, requires roots to grow deeper, especially in mixtures due to its overyielding of fine root production (Ma and Chen 2017). We thus hypothesized that positive mixture effects on turnover would shift to deep soil layers with stand development. Since fine root biomass loss from mortality is closely related to turnover rates (Persson 1980, Hendrick and Pregitzer 1993, Gill et al. 2002, Iversen et al. 2008), we also hypothesized that mixture effects on mortality would also shift to deep soil layers with stand development.

Species richness is commonly used as the measure of species diversity to define diversity effects on fine root dynamics in most studies (Ma and Chen 2016). However, richness alone cannot fully represent species diversity because it ignores the strong influence

of species evenness (relative abundance) on interspecific root interactions (Kirwan et al. 2007, Hillebrand et al. 2008). In accordance with the highly pronounced positive species evenness effects on both above- and below-ground productivity in forests (Zhang et al. 2012, Brassard et al. 2013, Ma and Chen 2017), we hypothesized that species evenness would have a strong positive effect on fine root turnover and mortality.

As the most common natural disturbance in boreal forests, wildfire has resulted in a diversity of forest mosaic ranging in composition from pure deciduous and mixed deciduous-coniferous to pure coniferous stands. Our previous study showed that species mixture effects on fine root productivity increases from 8- to 34-year-old post-fire stands (Ma and Chen 2017). Here, we examined species mixture effects on fine root turnover and mortality. We specifically tested whether (i) species mixtures would have higher fine root turnover and mortality than expected from those of single species-dominated stands, and the species mixture effects on turnover and mortality would increase with stand development; (ii) positive mixture effects on turnover and mortality would shift to deep soil layers with stand development; and, (Chapin III et al.) across a range of overstory compositions, fine root turnover and mortality would increase with species richness and evenness. We also expected that turnover and mortality rates would be positively associated with both fine root biomass and production because increased resource competition associated with high biomass and production reduces fine root longevity (Beyer et al. 2013, Brassard et al. 2013, Ma and Chen 2017).

4.3 Material and methods

4.3.1 Study area and experimental design

Our study area was located approximately 150 km north of Thunder Bay, Ontario, between 49°27' N to 49°38' N, and 89°29' W to 89°54' W. The mean annual temperature and annual precipitation between 1981 and 2010 was 1.9 °C and 824 mm, respectively, at the closest climatic station of Cameron Falls (Environment Canada 2016). The topographical features were shaped by the retreat of the Laurentide Ice Sheet approximately ten millennia ago. Soils are relatively deep glacial tills of the Brunisolic order on the upland sites (Soil Classification Working Group 1998). Wildfire is the primary stand-replacing natural disturbance in our study area, with an average fire return of approximately 100 years over the last century (Senici et al. 2010). Full details of the experimental design were described in Ma and Chen (2017). Here, a brief description is given.

We sampled two post-fire stand age classes (i.e., 8 and 34 years since fire) and three overstory types (single-species stands dominated by *Populus tremuloides* Michx. (*Populus*) and dominated by *Pinus banksiana* Lamb. (*Pinus*) to mixtures (*Populus+Pinus*)) at mesic sites in the study area. We replicated each of the stand age classes and overstory types three times. Stand ages were derived from fire records and verified by sampling dominant trees (Senici et al. 2010). Single- and mixed-species stands were defined as stands which contained a $\geq 80\%$ stand basal area of a single species and stands in which none of the component species had a $\geq 80\%$ stand basal area, respectively (Brassard et al. 2013, Ma and Chen 2017). Understorey vegetation accounts for the majority of species diversity and has strong affiliations with overstorey composition (Bartels and Chen 2013). We used ecological classification approach (Taylor 2000) to sample stands and ensured site similarity through a

comparison of the physical and chemical properties of soils. Stands were allocated several kilometers apart from each other to minimize neighborhood and unknown environmental influence that might be spatially correlated.

4.3.2 Data collection

A circular plot (400 m² in the 34-year-old stands, and 50 m² in the 8-year-old stands due to high stem densities) (Hart and Chen 2008) was randomly established to represent each sample stand. All live trees with diameter at breast height (DBH) over 2 cm were measured and recorded. The characteristics of our study stands were previously described in details (Ma and Chen 2017). Species richness was the number of tree species in the plot. We calculated Shannon's index (Shannon and Weaver 1949) using the species proportions based on their relative stand basal area (Table 4.1). Species evenness was calculated using J' index (Pielou 1969) as the ratio of Shannon's index to the natural logarithm of species richness. Similar to the natural stands studied by Brassard et al. (2013), there was higher species evenness in species mixtures than single species dominated stands, but no difference in species richness. Understorey plant cover was visually estimated for each species using the method described by Hart and Chen (2008). Because of the overlapping canopies among individual species, understorey vegetation cover could exceed 100% (Table 4.1).

We used sequential soil coring to determine fine root biomass, production, mortality, and turnover, following the method described previously (Yuan and Chen 2012, Brassard et al. 2013). Although ingrowth core method is frequently used in root studies, it produces substantially lower estimates of fine root processes than sequential coring method in natural forests, though qualitatively similar trends associated with stand development (Yuan and Chen 2012) and effects of species diversity (Brassard et al. 2013). In an undisturbed natural

Table 4.1. Shannon's index, forest floor depth and understorey vegetation cover (mean and 1 s.e.m., $n = 3$) of the study stands. Stand types are single-species *Pinus banksiana* dominated (*Pinus*), single-species *Populus tremuloides* dominated (*Populus*), and their mixtures (*Populus+Pinus*).

| Stand type | 8-years post-fire | | | 34-years post-fire | | |
|----------------------------------|----------------------|----------------------|-------------------|----------------------|----------------------|-------------------|
| | <i>Populus</i> | <i>Populus+Pinus</i> | <i>Pinus</i> | <i>Populus</i> | <i>Populus+Pinus</i> | <i>Pinus</i> |
| Shannon's index | 0.30 ± 0.18^{bc} | 0.83 ± 0.16^a | 0.12 ± 0.04^c | 0.29 ± 0.22^{bc} | 0.77 ± 0.21^{ab} | 0.13 ± 0.22^c |
| Forest floor depth | 1.89 ± 0.75^b | 1.52 ± 0.35^b | 2.76 ± 0.17^b | 6.33 ± 0.31^a | 6.76 ± 0.41^a | 7.34 ± 0.58^a |
| Understorey vegetation cover (%) | 116 ± 15^b | 101 ± 8^b | 102 ± 26^b | 135 ± 15^{ab} | 134 ± 7^{ab} | 170 ± 22^a |

Differences in characteristics among stand types and ages were tested using a two-way ANOVA. Different letters indicate a significant difference among stand age and type combinations ($\alpha = 0.05$)

ecosystem, soils have multiple and well-defined horizons. The manipulation of soil during root extraction and subsequent re-packing by ingrowth core method has been questioned for its suitability for studying fine roots in natural ecosystems (Mancuso 2011).

In each plot, we randomly extracted seven soil cores (6.6 cm in diameter) from the forest floor to a mineral soil depth of 30 cm, which accounts for approximately 90% of fine roots in boreal forests (Yuan and Chen 2010). We conducted root coring using a power auger monthly during 2014 growing season (May to October). Fine root production during the winter months was assumed to be negligible in the boreal forest (Steele et al. 1997, Konopka et al. 2005). Total sampling volume for each plot in this study was much higher than the recommended for root studies in natural systems (Taylor et al. 2013). We extracted soil samples by forest floor layer (FF) with its depth recorded on site, and two mineral soil sections: MS1 (0-15 cm) and MS2 (15-30 cm). Our sampling yielded a total of 2268 samples for laboratory analysis.

Samples were first soaked in water to separate roots from the soil, and then hand sorted to remove visible roots and coarse fragments. The remaining roots were isolated from the soil by rinsing with water over a 0.5 mm mesh screen. Fine roots ($\text{Ø} < 2$ mm, determined using calipers) were selected and further sorted according to their status (live versus dead), followed by the method described in Brassard et al. (2013). The 'live' and 'dead' root components were then separately oven-dried at 65 °C to a constant mass and weighed.

4.3.3 Statistical analysis

Fine root biomass (live roots) and necromass (dead roots) (Mg ha^{-1}) were calculated for each sampling date at each site by summing the dry weight of live and dead fine roots in each soil core and scaled up to per ha. We calculated fine root production ($\text{Mg ha}^{-1} \text{ year}^{-1}$) and

mortality ($\text{Mg ha}^{-1} \text{ year}^{-1}$) by determining all changes in dry weights for all sampling dates using a simplified decision matrix method (Yuan and Chen 2013), which was built upon a previously described matrix method (McClaugherty et al. 1982). Because all current methods have inherent strengths and weakness for estimating fine root production at the stand level (Vogt et al. 1998, Hendricks et al. 2006), we also used the MaxMin method to calculate annual production as the difference between the highest and lowest biomass observed over the entire measurement year (Edwards and Harris 1977). Given the qualitatively similar results obtained by these two methods (see Figure 4.1 and Figure 4.5), and that MaxMin method is conservative for temporal trends, we focused on reporting the results based on the simplified decision matrix method. Fine root turnover rate (year^{-1}) was defined as the ratio of annual production ($\text{Mg ha}^{-1} \text{ year}^{-1}$) over the mean standing biomass (Mg ha^{-1}) of fine roots (Aber et al. 1985).

We calculated the effects of species mixtures on turnover and mortality as the ratio (R_o) of the observed value to the expected value (the weighted average monoculture of the component species) of fine root turnover and mortality in mixtures (Loreau and Hector 2001). We calculated transgressive overyielding (R_t) as the ratio of the observed turnover or mortality in mixtures to that of the observed highest monocultures of the component species. We tested the effects of stand age on R_o and R_t of the turnover and mortality by the sum of all layers as well as by individual layers using a one-way analysis of variance (ANOVA). For individual layers, we standardized biomass turnover rates and mortality by soil depth on per cm basis due to variable depths of forest floor layer (Table 4.1). We tested the effect of stand age and types on fine root turnover and mortality using a two-way ANOVA. We also tested whether the effects of tree species richness, evenness, and Shannon's index on fine root

turnover and mortality differed among stand ages by examining the interaction term of the two-way ANOVA. To examine how turnover rate and mortality were related to annual production and average fine root biomass during the measurement year, we used type II regression analysis. We examined the assumptions of normality and homogeneous variance by Shapiro-Wilk's test and Leven's test, respectively, and these tests confirmed the assumptions were met for all analyses. All statistical analyses were conducted with R (version 3.3.2).

4.4 Results

Fine root biomass turnover and mortality were significantly higher in mixtures than expected from those of single species-dominated stands for both stand ages (Figure 4.1a). Although the magnitude of mixtures effect did not differ statistically between stand ages (Table 4.2), fine root turnover rates were 24% and 63% higher in the 8- and 34-year-old mixtures than the means of respectively aged single species-dominated stands (Figure 4.1a). Similarly, fine root mortality was 45% and 73% higher in the 8- and 34-year-old mixtures than the means of respective single species-dominated stands (Figure 4.1b). Fine root turnover and mortality were marginally (90% confident intervals) higher in mixtures than the more productive *Populus* stands in the 34-year-old stands, but not in the 8-year-old stands (Figure 4.1a,b). Fine root turnover rate and mortality differed among stand types with a significant interaction effect of stand type and stand age (Table 4.3). In the 8-year-old stands, turnover rate and mortality were highest in *Populus* stands, while the highest turnover rate and mortality occurred in the mixtures in the 34-year-old stands (Figure 4.1c,d).

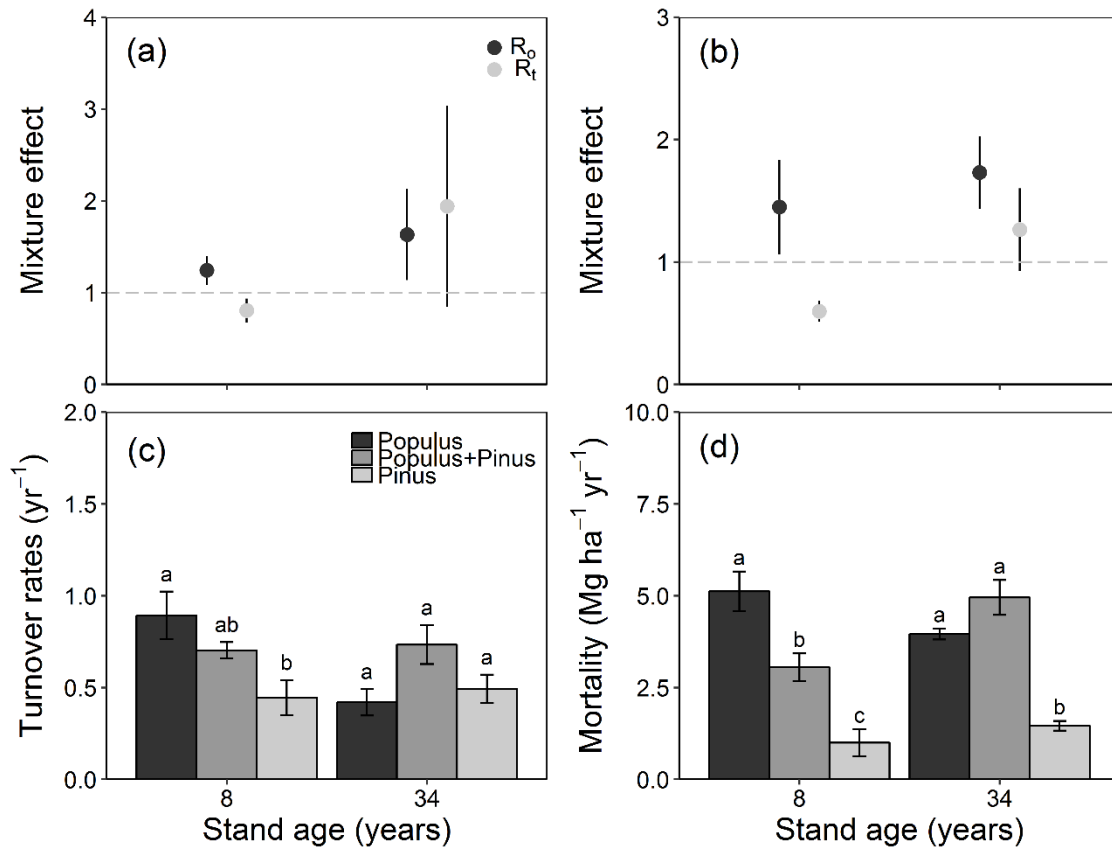


Figure 4.1. Effects of mixture on fine root turnover (a, c) and mortality (b, d) in relation to stand development. Mean and 95% confidence intervals of R_o and R_t higher than 1 indicate overyielding and transgressive overyielding of fine root turnover or mortality, respectively. Stand types were single-species *Populus tremuloides*-dominated (*Populus*), single-species *Pinus banksiana*-dominated (*Pinus*) and their even mixture (*Populus* + *Pinus*). Error bar represent 1 SEM ($n = 3$). Different letters indicate a significant difference between stands within the same age category ($\alpha = 0.05$).

Table 4.2. Effects of stand age on R_o and R_t for fine root turnover and mortality.

| Source | d.f. | $R_o(\text{turnover})$ | | $R_t(\text{turnover})$ | | $R_o(\text{mortality})$ | | $R_t(\text{mortality})$ | |
|-----------|------|------------------------|----------|------------------------|----------|-------------------------|----------|-------------------------|----------|
| | | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> |
| Stand age | 1 | 0.226 | 0.218 | 1.931 | 0.113 | 0.119 | 0.317 | 0.668 | 0.020 |
| Error | 4 | 0.106 | | 0.473 | | 0.091 | | 0.047 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (*P*).

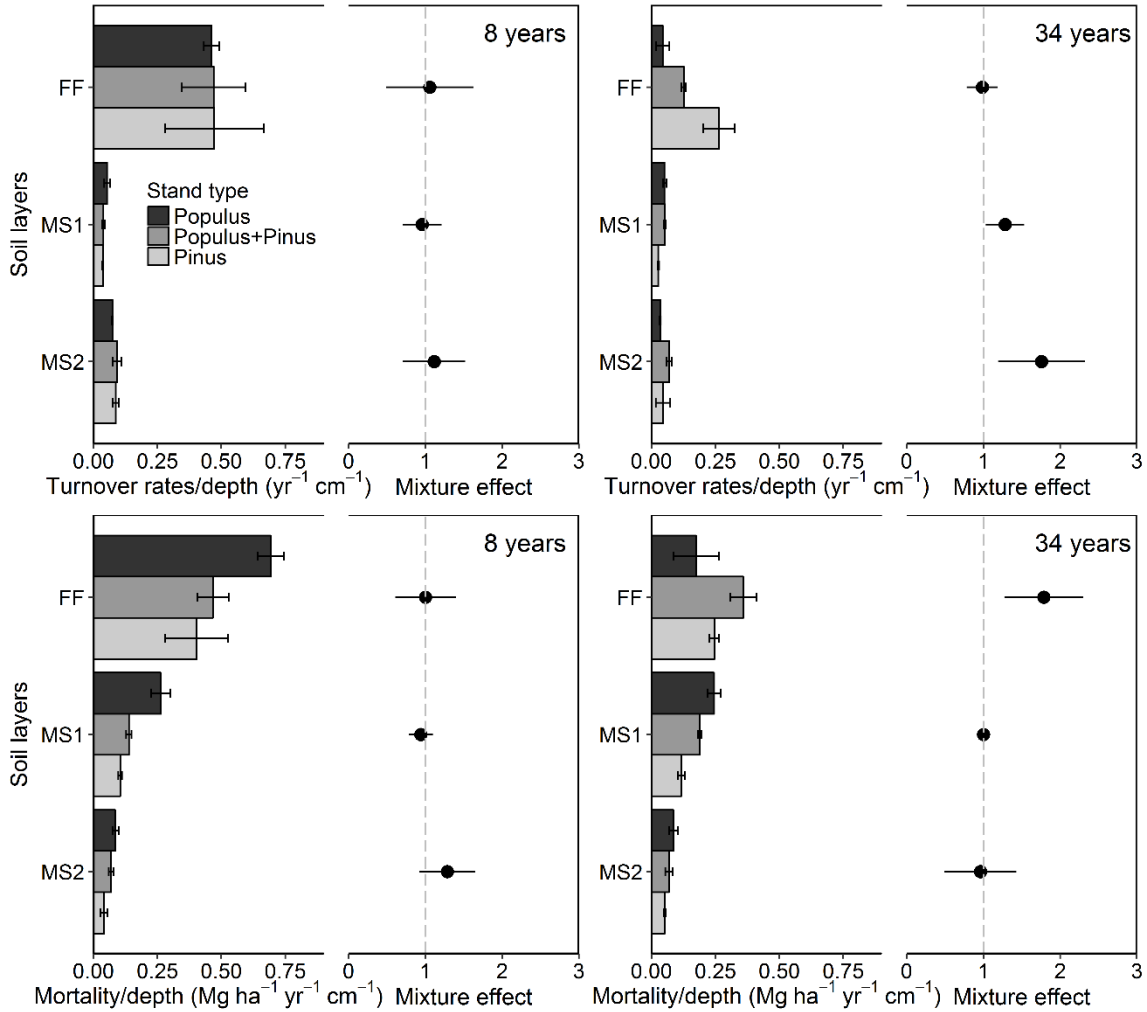


Figure 4.2. Effects of mixture on fine root turnover and mortality in relation to soil layers for each stand age. Turnover rates and mortality were standardized by soil depth on per cm basis due to variable depths of forest floor layer. Soil layers are forest floor (FF), mineral layer 1 (MS1, 0-15 cm) and mineral layer 2 (MS2, 15-30 cm). Mixture effects were calculated as overyielding, R_o mean and 95% confidence intervals. Stand types were described in Figure. 1. Error bar represent 1 SEM ($n = 3$).

Table 4.3. Effects of stand type and age on fine root turnover and mortality.

| Source | d.f. | Turnover | | Mortality | |
|------------------------|------|----------|----------|-----------|----------|
| | | MS | <i>P</i> | MS | <i>P</i> |
| Stand type | 2 | 0.101 | 0.045 | 18.96 | <0.001 |
| Stand age | 1 | 0.077 | 0.104 | 0.716 | 0.215 |
| Stand type × Stand age | 2 | 0.132 | 0.023 | 3.520 | 0.005 |
| Error | 12 | 0.025 | | 0.417 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (*P*).

Fine root biomass turnover and mortality (per cm soil depth) decreased with stand development in forest floor layers (Figure 4.2), whose depth increased with stand development (Table 4.1). In 8-year-old stands, the mixture effects on fine root turnover rate did not change with soil depth (Figure 4.2). In contrast, in 34-year-old stands, the mixture effects on fine root turnover rate increased with increasing soil depth with significantly positive effects in deep soil layers (Figure 4.2). Similarly, the mixture effects on fine root mortality did not differ with soil depth in the 8-year-old stands but decreased with soil depth in the 34 years-old stands (Figure 4.2).

Tree species richness did not have significant effects on fine root biomass turnover and mortality in either stand age class (Figure 4.3a, d; Table 4.4). However, biomass turnover and mortality significantly increased with tree species evenness for both overall and 34-year-old stands, but not in 8-year-old stands (Figure 4.3b, d). Similarly, fine root biomass turnover and mortality increased with Shannon's index for both overall and 34-year-old stands, but not in 8-year-old stands (Figure 4.3c, f, Table 4.4). Across all stand types and ages, fine root biomass turnover and mortality were positively associated with annual fine root production (Figure 4.4 a, c). Fine root mortality was positively correlated with fine root biomass (Figure 4.4 d), but there was no relationship between fine root turnover and biomass (Figure 4.4 b).

4.5 Discussion

Complementing our previous finding of positive mixture effects on fine root biomass and production (Ma and Chen 2017), we found that fine root biomass turnover and mortality were higher in species mixtures than expected from those of single-species dominated stands in both young and mature natural forests. Importantly, our results also highlight that the magnitude of these positive mixture effects increased with stand development.

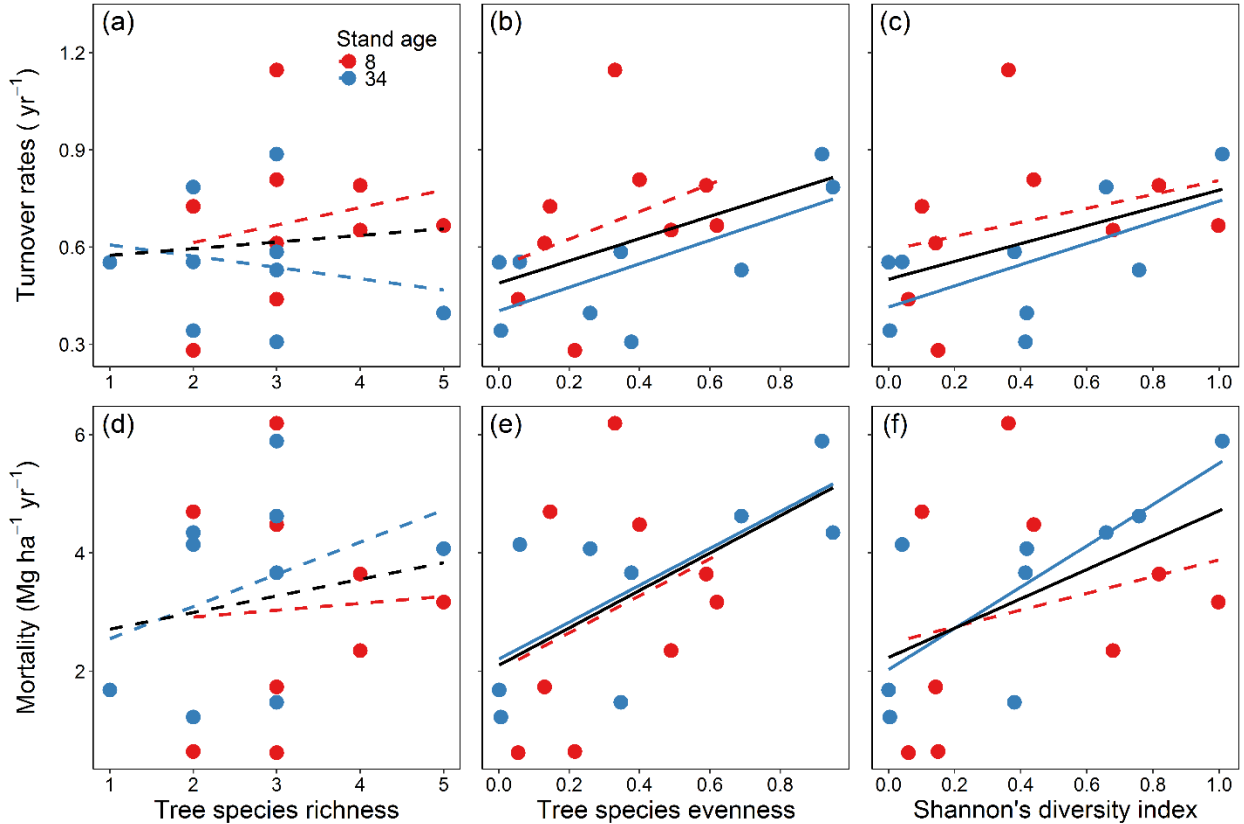


Figure 4.3. Fine root turnover (a-c) and mortality (d-f) in relation to tree species richness, evenness, and Shannon’s diversity index. 8- and 34-year-stands were represented by red and blue circles, respectively. Black lines represent overall response across two stand ages ($n = 18$). Solid lines represent significant relationships ($P < 0.05$), and dashed lines insignificant ($P \geq 0.05$).

In other words, when the two dominant boreal tree species (*Populus* and *Pinus*) are growing together, fine root biomass at the stand-level turned over faster and translocated more carbon from the mortality of fine roots to soil organic pool, especially in mature stands. Our results of positive mixture effects on fine root turnover are in agreement with findings in temperate forests (Lei et al. 2012a, Jacob et al. 2014). These results extend the evidence of positive mixture effects across a diverse range of ecosystem functions (Cardinale et al. 2012, Zhang et al. 2012, Duffy et al. 2017) to fine root turnover and mortality in natural forests.

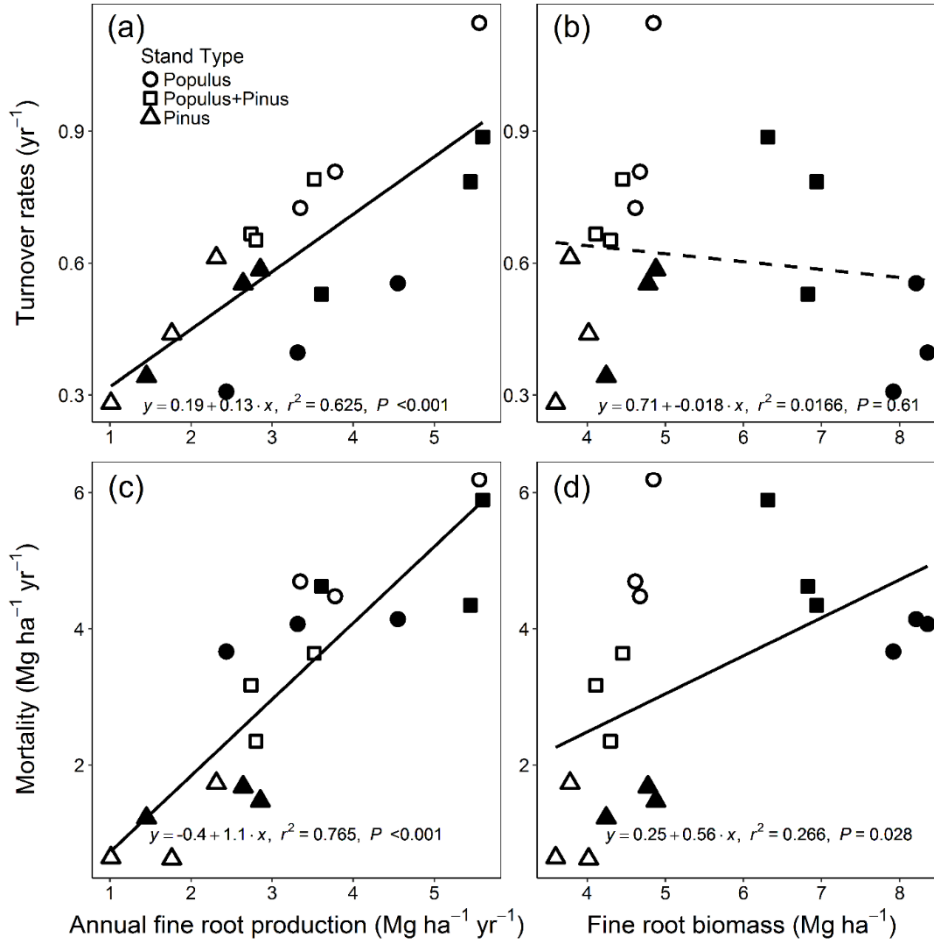


Figure 4.4. Pattern of increasing turnover rates and mortality with greater fine root production (a, c) and average standing fine root biomass (b, d) observed across stand types and ages. 8- and 34-year-stands were represented by open and closed shapes, respectively. Within each panel, the solid line represents a significant relationship through all three stand types and ages ($n = 18$), while the dashed lines represent an insignificant relationship. Reported P value and R^2 are shown for regression lines across all stand types.

Moreover, our results represent the first demonstration of increasingly positive mixture effects on fine root biomass turnover and mortality with stand development in natural forests, corroborating other evidence that positive mixture effects on ecosystem functions increase over time (Reich et al. 2012, Zhang et al. 2012).

Table 4.4. Effects of tree species richness, evenness, and Shannon’s diversity index (H) on fine root turnover and mortality of the entire soil profile among stand ages.

| Source | d.f. | Turnover | | Mortality | |
|----------------------|------|----------|----------|-----------|--------------|
| | | MS | <i>P</i> | MS | <i>P</i> |
| Richness | 1 | 0.008 | 0.702 | 1.496 | 0.515 |
| Stand age | 1 | 0.069 | 0.268 | 1.496 | 0.515 |
| Richness × Stand age | 1 | 0.034 | 0.434 | 0.789 | 0.635 |
| Error | 14 | 0.052 | | 3.350 | |
| Evenness | 1 | 0.174 | 0.055 | 14.75 | 0.031 |
| Stand age | 1 | 0.110 | 0.119 | 0.143 | 0.817 |
| Evenness × Stand age | 1 | 0.001 | 0.877 | 0.000 | 0.997 |
| Error | 14 | 0.559 | | 35.79 | |
| H | 1 | 0.148 | 0.087 | 12.04 | 0.047 |
| Stand age | 1 | 0.075 | 0.212 | 0.783 | 0.588 |
| H × Stand age | 1 | 0.006 | 0.714 | 2.104 | 0.379 |
| Error | 14 | 0.044 | | 2.554 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (P).

As we hypothesized, positive mixture effects on fine root biomass turnover shifted to deep soil layer with stand development. Plants in mixtures tend to grow more roots into deep soil layers with stand development (Ma and Chen 2017) and nutrients such as nitrogen and phosphorus decline abruptly with soil depth (Brady and Weil 1996). The higher biomass turnover in the deep soil layer in mature mixtures could be attributed to the increased root competition intensity caused by increased amount of competitors (fine root biomass) per unit of available nutrients. By contrast, mixture effects on fine root mortality decreased with increasing soil depth in mature stands. The high root mortality observed in forest floor layer of the 34-year-old mixtures could probably be attributed to its higher root production as well as shading effects (stem exclusion stage) on small trees and understory plants (Chen and Popadiouk 2002a), as they mainly root in the forest floor (Chen and Brassard 2013). The greater fine root production in the deep soil

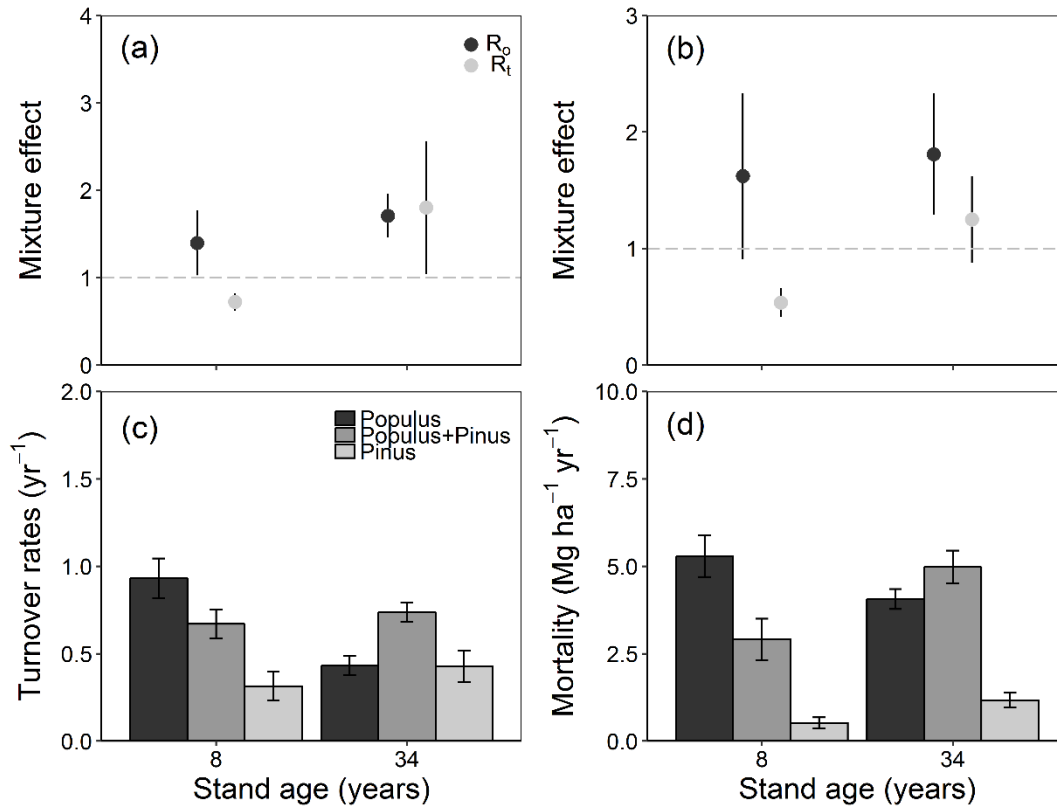


Figure 4.5. Effects of mixture on fine root turnover (a, c) and mortality (b, d) in relation to stand development based on MaxMin method. Mean and 95% confidence intervals of R_0 and R_f higher than 1 indicate overyielding and transgressive overyielding of fine root turnover or mortality, respectively. Stand types were described in Figure. 1. Error bar represent 1 SEM ($n = 3$).

layer in the 34-year-old mixtures (Ma and Chen 2017) without increased mortality could result from increasing dominance of tree roots that have longer lifespans than shrub and herb roots (Chen and Brassard 2013).

Neither richness nor evenness significantly affected fine root biomass turnover and mortality in young stands. However, in mature stands, biomass turnover and mortality increased with evenness, but not with richness. One of the possible explanations for the positive effect of evenness on biomass turnover and mortality is that evenness can increase competition intensity by affecting the relative strength of interspecific interactions within

communities (Hillebrand et al. 2008). The positive effect of evenness found in our study is consistent with previous findings that evenness positively affect both above- and below-ground productivity in forests (Zhang et al. 2012, Brassard et al. 2013, Ma and Chen 2017), highlighting the important role of species evenness in biodiversity and ecosystem function relationships (Hillebrand et al. 2008). Future studies should place a particular emphasis on the important role of evenness in biodiversity and ecosystem function studies, particularly in natural systems where evenness varies substantially (Zhang et al. 2014).

As expected, we found that fine root biomass turnover rate and mortality were positively associated with annual fine root production across all stand types and ages. This agrees with the finding of a recent study showing increased turnover rates with greater total annual root production across 12 temperate species (McCormack et al. 2014). One possible explanation for the positive relationship between fine root turnover and production is that higher fine root biomass production leads to greater resource scarcity and root competition which eventually decreases root longevity (Beyer et al. 2013). Our results indicate that stand-level annual root production can be a useful predictor for stand-level fine root turnover and biomass loss through mortality, at least for *Populus* and *Pinus*. More importantly, these findings further suggest that the observed increase in mixture effects on biomass turnover and mortality with stand development in our study might have resulted from the increased competition intensity induced by the overyielding of fine root biomass production, previously reported by Ma and Chen (2017). We note that, however, this may not be universal as evidence in the deep soil layer of the 34-year-old mixtures. Our results also revealed that average standing fine root biomass was positively associated with fine root mortality but not with biomass turnover, indicating that annual fine root production may be a better predictor of

biomass turnover and mortality than standing biomass. While current technologies allow using traditionally defined fine root (≤ 2 mm in diameter) to study stand-level fine root demographics, future studies could build on the proposed root functional approach (McCormack et al. 2015, McCormack et al. 2017) to better understand how species diversity impacts belowground processes at the ecosystem level.

4.6 Conclusion

In summary, by studying the mixture effects on fine root biomass turnover and mortality in a natural boreal forest, we provide direct evidence of increasing positive mixture effects on root turnover and mortality with stand development. Moreover, we found that mixture effects shift among soil layers with stand development and the increased mixture effects with stand development might have resulted from the increased competition intensity induced by the overyielding of fine root biomass production. Our results extend the understanding of diversity effects on ecosystem function relationships to fine root turnover and mortality in natural forests and contribute to model terrestrial biogeochemistry.

CHAPTER 5: EFFECT OF SPECIES MIXTURES ON PRODUCTION PARTITIONING ALONG STAND DEVELOPMENT IN A NATURAL BOREAL FOREST

5.1 Abstract

Most published diversity and productivity relationship (DPR) studies focus on one component of ecosystem production. Species diversity could alter production allocation, and at least, in part, contribute to divergent DPR relationships. By synthesizing the production data of all individual components (i.e., aboveground trees, litterfall, understory vegetation, coarse roots, and fine roots) of natural boreal forest stands, collected from the same study sites, we examined how species mixtures affected the production of the entire ecosystem, and production allocation among individual components along stand development. We found that overyielding of the entire ecosystem production occurred in young, but not older stands, despite the fact that fine root production was higher in species mixtures than single-species dominated stands in all ages. Species mixtures led to more production allocated to belowground than was expected from single species-dominated stands. Both production and its allocation were significantly affected by the availability of soil nutrients. Our study offers a new and critical elucidation of DPR, by showing the temporal change of mixture effects on ecosystem production and its allocation in natural forests. The results have relevance for calculating the allocation of energy, as well as carbon storage in terrestrial ecosystems.

5.2 Introduction

Species extinctions on a global scale are altering Earth's ecosystems (Hooper et al. 2012). In recent decades, many diversity and productivity relationships (DPRs) studies designed across biomes (primarily in controlled experiments), have observed positive relationships (Tilman et al. 1996, Loreau and Hector 2001, Cardinale et al. 2007, Liang et al. 2016). Through the synthesis of 67 field studies, a recent meta-analysis indicated even stronger diversity effects on productivity in natural ecosystems (Duffy et al. 2017). However, positive DPRs are far from certain (Adler et al. 2011, Fraser et al. 2015) as the vast majority of these studies separately tested diversity effects on certain component of production (e.g., aboveground biomass and fine roots) (Zhang et al. 2012, Ma and Chen 2016), with only few experimental studies that considered total biomass production in grasslands (Tilman et al. 2001, Reich et al. 2012). How productivity responds to diversity from the whole ecosystem perspective in natural ecosystems is poorly understood, particularly in natural forests characterized by the dominance of long-lived organisms (trees) and high level of structural complexity and environmental heterogeneity (Leuschner et al. 2009).

Ecosystem functions and services provided by forests reflect the contributions of all components. Field measurements of net primary production (NPP) can be partitioned into several individual parts (Chapin III et al. 2011) (Table 5.1): Estimates of aboveground tree production (ATP) through the exclusive use of large sized trees, are typically biased (Searle and Chen 2017). Understory vegetation production (UP), which is often excluded when estimating aboveground production, can account for a substantial proportion of NPP in forests (Chapin III et al. 2011). Litterfall production (LP) is a major component of NPP, and its contribution can increase with stand age in boreal forests (Chen et al. 2017). Coarse root

(CRP) and fine root production (FRP), which significantly contribute to NPP (Jackson et al. 1997, McCormack et al. 2015), are also altered with stand development (Yuan and Chen 2012). Previous empirical diversity and production relationship (DPR) studies in forests, primarily centred on aboveground trees, with a few focusing on other components (i.e., understorey vegetation and fine roots) have reported inconsistent results (Pretzsch and Schutze 2009, Cavard et al. 2010, 2011a, Gamfeldt et al. 2013, Domisch et al. 2015, Zhang et al. 2016, Ma and Chen 2017). These divergent findings may arise due to potential shifts in the allocation of production among these components in mixtures (Epron et al. 2013), as well as along stand development (Litton et al. 2007). The lack of understanding of potential diversity effects on production allocation, and its changes along stand development, hampers the complete appreciation of diversity effects on ecosystem productivity. For example, can the overyielding of wood production in mixtures be mostly explained by the increased total ecosystem NPP, or by shifts in the fraction of NPP that is employed for aboveground wood production?

Table 5.1. Classification of five components of production ($\text{Mg ha}^{-1} \text{ year}^{-1}$) in natural forests.

| | Component | Description |
|-------------------------------|--|--|
| Aboveground production (ANPP) | Aboveground tree production (ATP) | All trees with height ≥ 1.3 m |
| | Overstorey trees | Canopy and emergent trees |
| | Understorey trees | Trees underneath canopy with height ≥ 1.3 m |
| | Understorey vegetation production (UP) | Trees with height < 1.3 m and non-tree vegetation |
| | Shrubs | Woody plants with height < 1.3 m |
| | Herbs | Non-woody vascular plants |
| | Bryophytes | Ground-growing non-vascular plants |
| | Litterfall production (LP) | Annual production of litter dry mass |
| Belowground production (BNPP) | Coarse root production (CRP) | Annual production of coarse roots (≥ 2 mm in diameter) |
| | Fine root production (FRP) | Annual production of fine roots (< 2 mm in diameter) |

It has been theorized that plants allocate production to minimize resource limitation and maximize resource capture and NPP, and production allocation changes with ontogeny, resource availability, and climatic conditions (Mokany et al. 2006, Litton et al. 2007, Poorter et al. 2012). Based on this concept, production allocation in mixtures is expected to be modified by interspecific interaction because all factors known to affect production allocation can be different in mixtures, in contrast to monocultures (Richards et al. 2010, Forrester 2014). Studies that compared production allocation patterns in mixtures and monocultures have been limited to a few young tropical forest plantations (Forrester et al. 2006, Nouvellon et al. 2012, Epron et al. 2013). Greater aboveground production allocation in mixtures can result from high soil N and P availability (Forrester et al. 2004), while increased allocation to belowground can be associated with water limitation that can dampen the potential benefits of N₂-fixing tree species in mixtures (Nouvellon et al. 2012). Compared with forest plantations, natural forests have a higher degree of structural complexity, environmental heterogeneity (Leuschner et al. 2009), and resource limitations, as well as natural successional processes. Despite its fundamental importance to DPRs studies, how mixtures affect production allocation in natural forests, and how they may shift along stand development remain unknown.

In natural forests where light and nutrients are limited, mixtures may affect production allocation through the complementary use of resources. For example, mixtures may increase aboveground production allocation through the complementary use of light among crowns in the canopy space (Williams et al. 2017). Mixtures may also increase allocation to fine roots to increase water and nutrient uptake, via increased root density per unit soil volume (de Kroon 2007) and increased soil volume filling (Brassard et al. 2013, Ma and Chen 2017). Production

allocation to above- and belowground may depend on which resources are more limiting (Bloom et al. 1985). The complementary use of light through stratification is common, as mixed-wood stands are typically more structurally diverse than single-species dominant stands in forests (Kelty 1989, MacPherson et al. 2001, Brassard and Chen 2008). However, complimentary use, or the increased use efficiency of soil resources through root segregation between coexisting species, appears to be the exception rather than the rule (Casper et al. 2003, De Kroon et al. 2003), since the roots of different species possess an equivalent probability of encountering, accessing, and utilizing soil resources (Cahill and Casper 2000, Schenk 2006). Moreover, because soil nutrients such as nitrogen (N) and phosphorus (P) are limiting for production in terrestrial ecosystems, likely more so in species mixtures due to their high production (Elser et al. 2007), we hypothesized that mixtures would allocate more production to fine roots to increase soil resource uptake.

The positive effects of production allocation to belowground may increase with stand development. According to the ontogenetic drift theory (Gedroc et al. 1996), production allocation shifts from below- to aboveground over the course of plant development. This shift appears to coincide with increasing soil N and P, and decreasing light availability with stand development in natural post-fire forests (Duran et al. 2008, Hume et al. 2016). However, since the structural diversity of live trees increases with stand development (Brassard and Chen 2008), the complementary use of light via canopy stratification would be expected to increase with stand development in species mixtures (Yachi and Loreau 2007, Williams et al. 2017). On the other hand, increased total plant biomass and production, particularly in species mixtures, would require additional investment in fine roots to increase soil resource uptake

with stand development. We therefore hypothesized that the positive effects of mixtures on belowground production allocation would increase with stand development.

Here, we examined the production of individual components, the ecosystem as a whole, and partitioning, in 8-, 34-, and 85-year-old single species-dominated and mixed post-fire stands in boreal forests. We specifically tested the hypotheses that: (1) belowground production allocation would be higher in species mixtures than expected from single-species dominated stands, and these mixture effects would increase with stand development. To further elucidate the potential mechanisms associated with allocation shifts, we examined the patterns of soil nutrients associated with stand development and their relationships with production allocation.

5.3 Materials and methods

5.3.1 Study area and experimental design

This study was carried out in the boreal forest located approximately 150 km north of Thunder Bay, Ontario, between 49°27' N to 49°38' N, and 89°29' W to 89°54' W. This region is characterized by a moderately dry and cool climate, with short summers. The closest meteorological station is located in Cameron Falls. The mean annual temperature and precipitation from 1981 to 2010 was 1.9 °C and 824 mm, respectively (Environment Canada 2016). The topographical features of this region were shaped by the retreat of the Laurentide Ice Sheet approximately ten millennia ago. The soils on the upland sites are relatively deep glacial tills belonging to the Brunisolic order (Soil Classification Working Group 1998). Fire is the most common natural disturbance in the study area with an average fire-return interval of approximately 100 years over the past century (Senici et al. 2010).

On mesic sites in the study area, which support a wide range of forest compositions due to the variation of local propagule availability (Ilisson and Chen 2009), we sampled three post-fire stand age classes (i.e., 8, 34, and 85 years since fire) and three overstory types (single-species stands dominated by *Populus tremuloides* Michx. (*Populus*), dominated by *Pinus banksiana* Lamb. (*Pinus*), and mixtures (*Populus+Pinus*)). Each of the stand age classes and overstory types was replicated three times, resulting in a total of 27 stands. Stand ages were determined from fire records and verified by sampling dominant trees (Senici et al. 2010). Single- and mixed-species stands were defined as stands that contained a $\geq 80\%$ stand basal area of a single species, and stands in which none of the component species had a $\geq 80\%$ stand basal area, respectively (Brassard et al. 2013, Ma and Chen 2017). Field work associated with the 8- and 34-year-old stands was conducted during 2013-2014, whereas that for the 85-year-old stands was performed during 2007-2008 (Brassard et al. 2013). Stands were allocated several kilometers apart from each other to minimize neighborhood and unknown environmental influences that might be spatially correlated. We employed an ecological classification approach (Taylor 2000) to sample stands, and ensured site similarity through a comparison of the physical and chemical properties of the soils. Moreover, the similarity of the sites was validated by soil textures of the mineral layer at a depth of 30–50 cm; no significant differences could be detected among stand age classes or composition types (Hume et al. 2016).

5.3.2 Data collection

A circular plot (400 m²) was randomly established to represent each sample stand.

Aboveground NPP was partitioned into overstorey tree layers, understorey tree layers, and understorey vegetation layers (i.e., shrubs, herbs, or bryophytes) based on vertical strata and

plant growth forms (Zhang et al. 2016), while belowground NPP was grouped as coarse roots and fine roots (Table 5.1).

Aboveground overstorey and understorey tree production (ATP)

Within each plot, overstorey and understorey trees were classified based on their crown positions in a stand (Avery and Burkhart 2002). In the 34- and 85-yr-old stands, overstorey trees coincided with trees with a diameter at breast height (d.b.h., 1.3 m above root collar) \geq 9.0 cm, and in the 8-yr-old stands, all trees with \geq 1.3 m in height were a part of the canopy. Stand basal areas, by overstorey tree layers, were summed to the plot level and used for assigning stand-type classification (Table S5.1). Species richness was the number of species in the plot. We calculated Shannon's index (Shannon and Weaver 1949) using the proportions of species based on their relative stand basal area. Species evenness was estimated using J' index (Pielou 1969), as the ratio of Shannon's diversity to the natural logarithm of species richness (Table S1). Understorey trees were measured within a 50 m² circular subplot (3.99 m radius; shared plot center with the main plot). The aboveground biomass of all trees \geq 3 cm in d.b.h. was estimated as the sum of bark, stem, branch, and foliage biomass calculated by using published Canadian allometric equations (Lambert et al. 2005). For tree saplings of $<$ 3 cm and \geq 1.3 m in height, the aboveground biomass was estimated by using allometric equations that were developed for small trees (Smith and Brand 1983, Bond-Lamberty et al. 2002a). Woody tissue samples were collected to estimate the annual biomass increment; full details can be found in Gao et al. (2016).

The annual production of trees (i.e., annual biomass increment) was calculated based on an average of the previous five years (2008 – 2013), using the method described by Chen and Klinka (2003). In brief, we initially developed the relationship between bark and stemwood

d.b.h. from the disk and core samples. Subsequently, we used that relationship to calculate d.b.h. with bark from measured stemwood d.b.h. in 2008 and 2013. Aboveground tree biomass in 2008 and 2013 was estimated from d.b.h. using allometric equations. Mean annual biomass production over the previous five years was $(2013 \text{ biomass} - 2008 \text{ biomass}) / 5$.

Litterfall (LP) and understorey vegetation production (UP)

Chen et al. (2017) provides full details of litterfall data collection. In brief, annual litterfall production was calculated as $\text{Mg ha}^{-1} \text{ y}^{-1}$ by summing all oven dried litterfall collections (leaves, needles, arboreal epiphytic lichens, and other materials) over an entire calendar year to represent annual leaf production. To sample the biomass and production of the shrub layer (Table 5.1), three 2×2 m subplots were randomly established within each plot. The height, stem diameter of individual shrubs, and tree saplings were numbered, tagged, and measured for two consecutive years. The biomass of the shrub layer was estimated using allometric equations (Smith and Brand 1983, Bond-Lamberty et al. 2002b) (scaled up to Mg C ha^{-1}) over two consecutive years, where annual production was the difference between the two years. Biomass production included biomass gain by the growth of surviving individuals, ingrowth by new recruits, and biomass loss due to mortality. For herbaceous plants and non-vascular species, one 2×2 m subplot was also randomly established in each plot, where the percentage of cover and height for all herbaceous and non-vascular plants was recorded. In order to estimate the biomass of herbaceous plants, we established a relationship between biomass and cover, and height for each individual species. Because aboveground parts of herbaceous species die annually, production was considered as equal to their peak biomass, in August of 2016.

Fine root production (FRP), and coarse root production (CRP)

Detailed information concerning fine root collection can be found in (Ma and Chen 2017). In brief, seven soil cores (6.6 cm in diameter) were randomly extracted from the forest floor to a mineral soil depth of 30 cm, using a power auger, every month during the growing season (May to October) in each of the 27 plots. Samples were initially soaked in water to separate roots from the soil, and then hand sorted to remove visible roots and coarse fragments. The remaining material was further gently rinsed over a sieve (0.5 mm mesh size) to remove the remaining root fragments. Fine roots ($\text{\O} < 2$ mm, as determined using calipers) were selected and further sorted according to their status (live versus dead). The ‘live’ and ‘dead’ root components were then separately oven dried to a constant mass at 65 °C and weighed.

Fine root biomass (live roots) and necromass (dead roots) (Mg ha^{-1}) were calculated for each sampling date at each site by summing the dry weight of live and dead fine roots in each soil core, and scaling up to per ha values. Estimates of fine root production ($\text{Mg ha}^{-1} \text{ year}^{-1}$) were calculated by determining all changes in the dry weights for all sampling dates, using a simplified decision matrix method. The coarse root biomass of all trees ≥ 9 cm in d.b.h. was calculated by using published allometric equations for large trees (Brassard et al. 2011a). For tree saplings and large shrubs < 9 cm, but ≥ 1.3 m in height, coarse root biomass was estimated by using allometric equations that were developed for small trees and large shrubs (Smith and Brand 1983, Ruark and Bockheim 1987, Bond-Lamberty et al. 2002b, Xing et al. 2005, Ouimet et al. 2008). Akin to aboveground tree biomass production, coarse root production was $(2013 \text{ biomass} - 2008 \text{ biomass}) / 5$.

Soil nutrients

Details regarding the determination of soil nutrients have been previously reported (Hume et al. 2016). In brief, ten soil samples (each divided into three layers), were randomly collected

within each plot and transported to the laboratory for chemical analysis. Soil samples were air-dried and passed through 2-mm sieve to remove stones and 0.15mm sieve to ensure the uniformity. Total nitrogen (N) concentration was measured using the dynamic flash combustion method, while total phosphorus (P) was determined via nitric/hydrochloric acid digestion method and inductively coupled plasma atomic emission spectrometry (ICP-AES). Total potassium (K), calcium (Ca) and magnesium concentration were determined by atomic absorption spectrophotometry (Perkin Elmer 5100 PC, Boston, MA, USA). Concentrations were measured as g kg^{-1} .

5.3.3 Statistical analysis

We calculated aboveground NPP (ANPP) and belowground NPP (BNPP) as:

$$\text{ANPP} = \text{ATP} + \text{LP} + \text{UP} \quad (1)$$

$$\text{BNPP} = \text{CRP} + \text{FRP} \quad (2)$$

Following Poorter et al. (2012), we calculated the production fractions for trees (ATPF), understory vegetation (UPF), litterfall (LPF), coarse roots (CRPF), and fine roots (FRPF), as the proportion of their production against total ecosystem production, i.e., the sum of all individual components. The aboveground and belowground production ratio (ANPP:BNPP) was also employed to represent the production allocation strategy. Species mixture effects on production and production fractions were calculated as the ratio (R_o) of the observed value to the expected value (the weighted average monoculture value of the component species) of production and production fraction in mixtures (Loreau and Hector 2001).

We tested whether the mixture effects on the production of individual components, and production fractions changed with stand age, by one way analysis of variance (ANOVA). We used principal component analysis (PCA) to identify the principal components of soil nutrients, since several soil nutrients were highly correlated. To examine how production and production allocation were impacted by the primary soil nutrient components, we used multiple regression analysis. The effects of stand age on the main soil nutrient components were also tested using one-way ANOVA. We examined the assumption of normality of homogeneous variance by Shapiro-Wilk's test and Leven's test, respectively; these tests confirmed that the assumptions were met for all analyses. All statistical analyses and graphs were performed and created, respectively, using the R Program for statistical computing and graphic display (R Core Development Team, 2017).

5.4 Results

The effects of mixtures on total ecosystem production were significantly positive in the 8-year-old stands, with 24% higher production in mixtures than expected from those of single species-dominated stands (Figure 5.1). However, mixtures did not significantly impact total ecosystem production in the 34- and 85-year-old stands (Figure 5.1). Stand age associated changes in species mixture effects on production varied among individual components. Mixtures had significantly negative effects on aboveground tree and understorey vegetation production in the 34- and 85-year-old stands, respectively (Figure 5.1). Mixture effects on fine root production were significantly positive in the 8- and 34-year-old stands, and marginally ($P = 0.29$) positive in the 85-year-old stands (Figure 5.1). Mixture effects were not statistically significant for litterfall and coarse root production for any age class (Figure 5.1).

Mixture effects were significantly affected by stand age only in the case of understory vegetation production (Table S5.2).

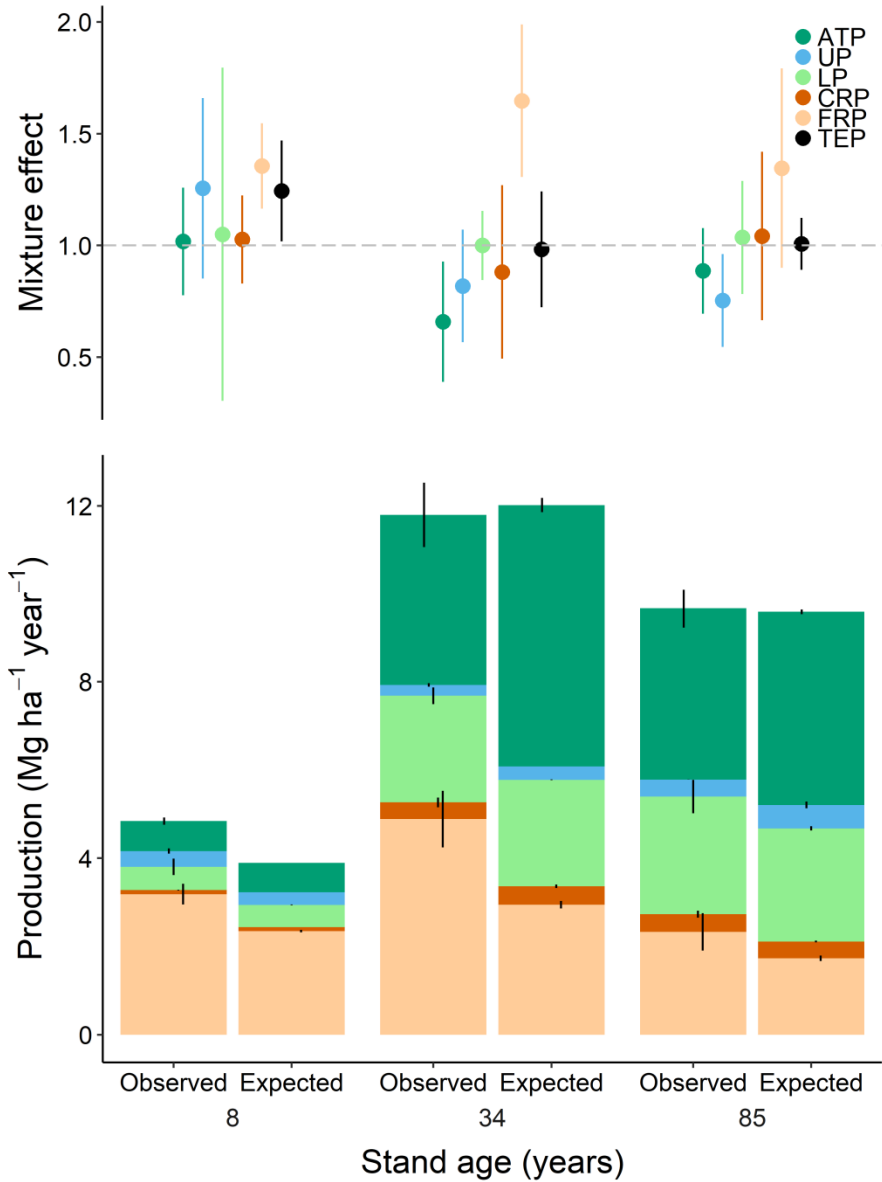


Figure 5.1. Effects of mixture on production of each individual component and entire ecosystem (upper panel), expected and observed values of production for the mixtures (lower panel), in relation to stand development. Mixture effects are represented by scatter with mean and 95% confidence intervals. Dark green: aboveground trees; light blue: understory vegetation; light green: litter fall; vermilion: coarse roots; orange: fine roots; black: entire ecosystem. Error bar represent 1 SEM ($n = 3$).

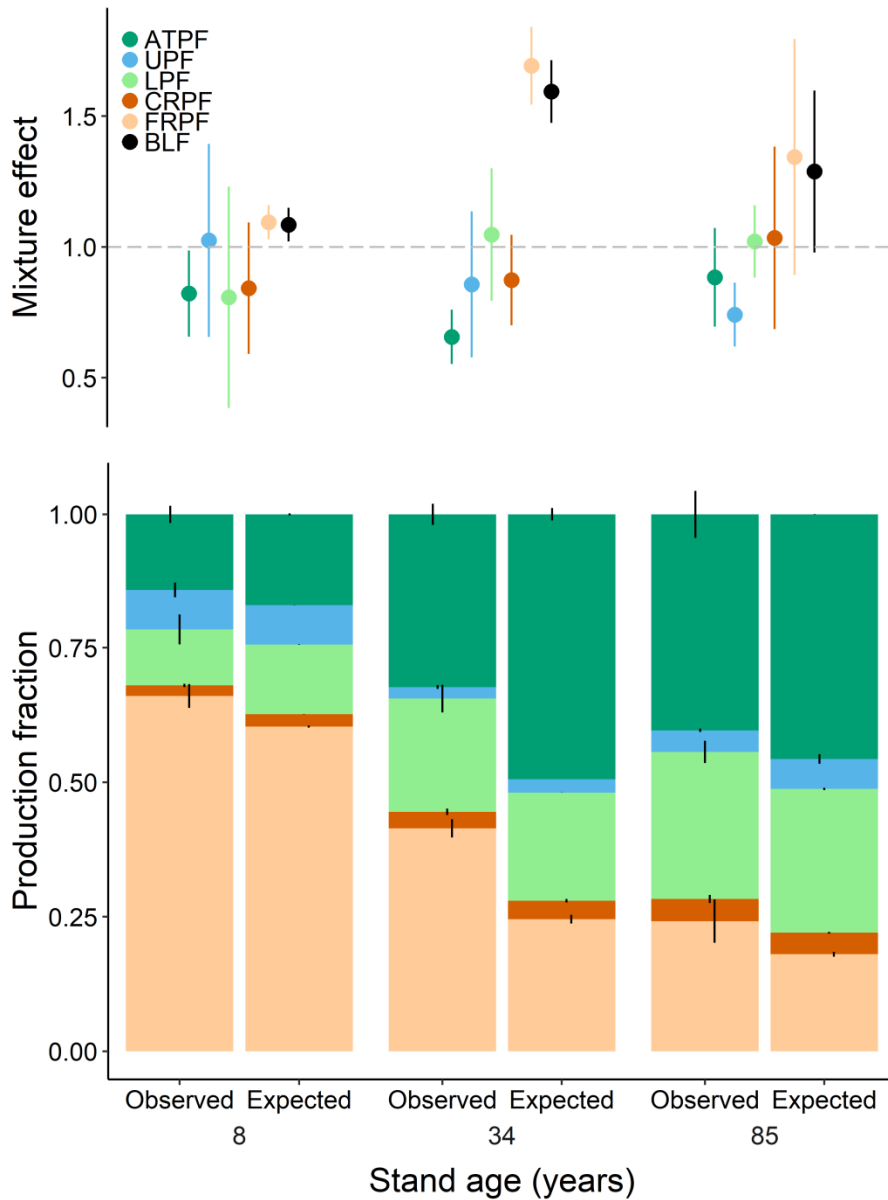


Figure 5.2. Effects of mixtures on production fraction of each individual component, and total belowground (upper panel), expected and observed values of production fraction for the mixtures (lower panel) in relation to stand development. Mixture effects are represented by scatter with mean and 95% confidence intervals. Dark green: aboveground trees; light blue: understory vegetation; light green: litter fall; vermilion: coarse roots; orange: fine roots; black: total belowground. Error bar represent 1 SEM ($n = 3$).

Along with stand development, mixtures allocated 8%, 56%, and 27% more production to belowground than expected from single species-dominant stands in the 8-, 34-, and 85-year-old stands, respectively (Figure 5.2; Table S5.3). Stand age associated changes in species mixture effects on the production fraction varied among individual components. Mixtures increased production allocation to fine roots across all three age classes, while reduced production allocated to aboveground trees was significant in both the 8- and 34-year-old stands, and the production fraction of understory vegetation in the 85-year-old stands (Figure 5.2). Increased production allocation to fine roots was significantly more pronounced in the 34-year-old stands than for the other stands (Figure 5.2, Table S5.3).

The first two PCA axes accounted for 92.5% of the variation of soil nutrients (Table 5.2), with the contents of N, P, K, Ca, and Mg being positively correlated with PC1, whereas those of N and Mg negatively correlated with PC2. PC1 and PC2 differed significantly among stand ages (Figure 5.3). Aboveground tree, litterfall, coarse root, and total ecosystem production increased with PC1; however, fine root and understory vegetation production did not change with PC1 (Figure S5.1, Table S5.4). The biomass production of aboveground trees, as well as the entire ecosystem increased significantly with PC2 (Figure S5.1, Table S5.4). For production allocation, PC1 significantly increased the fraction of production that was allocated to aboveground trees, litterfall, and coarse roots, while decreased fraction allocated to fine roots (Figure 5.4, Table S5.5). The fraction of production allocated to each component was not significantly affected by PC2 (Figure 5.4, Table S5.5). The ANPP:BNPP ratio was significantly positively affected by PC1, while it was not significantly affected by PC2 (Figure 5.4).

5.5 Discussion

Our results revealed that the nature of the mixture effects on total ecosystem production, as well as production allocation among individual components, changes with stand development

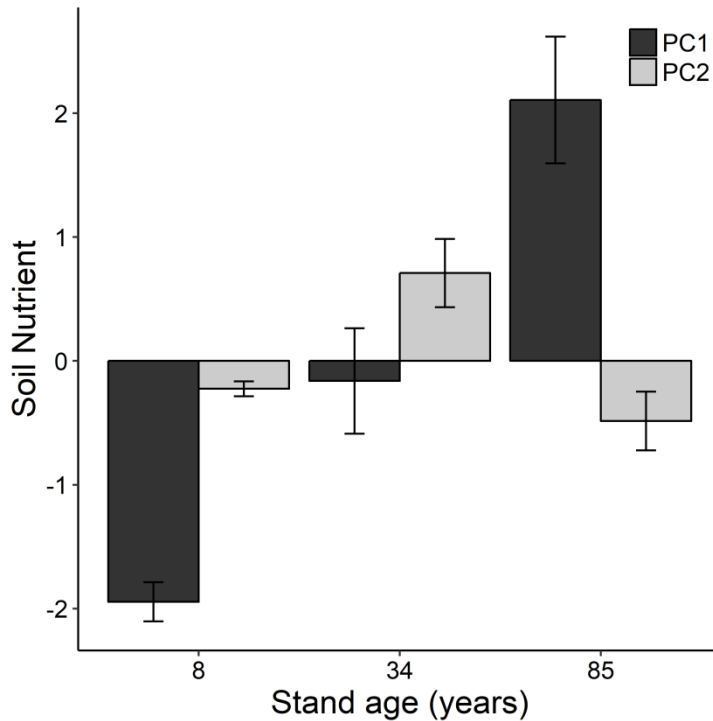


Figure 5.3. The first two principal components of soil nutrients change with stand development. Error bar represent 1 SEM ($n = 3$).

Table 5.2. The associations between soil nutrients and the first two PCA axes. The two PCA axes accounted for a total of 92.5% of the variation in soil nutrients (79.9% and 12.5%, respectively).

| | N | P | K | Ca | Mg |
|-----|-------------|-------------|-------------|-------------|-------------|
| PC1 | 14.8 | 21.5 | 22.7 | 23.6 | 17.3 |
| PC2 | 56.6 | 0.6 | 4.3 | 0.3 | 38.3 |

The values in bold face indicate strong loadings on each axis.

in forest ecosystems. Further, our results indicated that the observed stand age-dependent dynamics of production and their allocations are driven, in part, by changes in the nutritional properties that are associated with stand age.

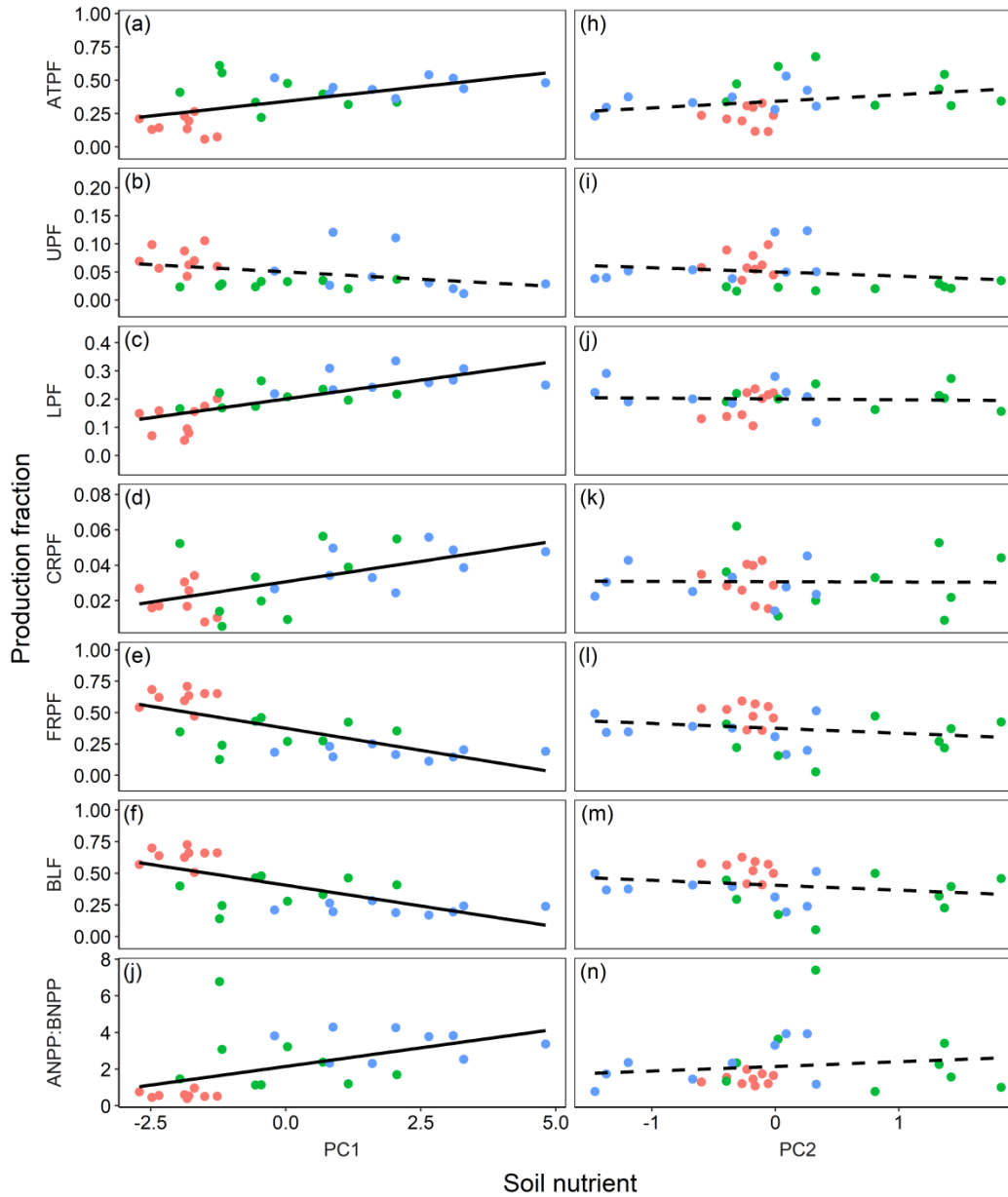


Fig. 4. Partial regression plots show the production allocation in relation to the first two principal components of soil nutrients (PC1 and PC2). Colours indicate stand age classes: red, 8 years; green, 34 years; blue, 85 years. Within each panel, the solid line represents a significant relationship through all three stand types and ages ($n = 27$), while the dashed lines represent an insignificant relationship.

Mixture effects on biomass production along stand development

Our results demonstrated a significant positive effect of tree species mixtures on productivity of entire ecosystems in natural forests of young stand ages. Importantly, however, our results also highlighted that these positive mixture effects vanished with stand development. The positive mixture effects observed in this study confirmed what has previously been reported for grasslands, showing positive diversity effects on total biomass production (Tilman et al. 2001, Reich et al. 2012). However, the disappearance of the mixture effects in mature and old stands were in contrast to earlier studies, which demonstrated an increased diversity effect over time (Tilman et al. 2001, Cardinale et al. 2007, Reich et al. 2012). Consistent with earlier studies conducted on grasslands, which demonstrated that diversity effects on root biomass lagged behind the effect on aboveground biomass (Ravenek et al. 2014), the response of biomass production to species mixture and stand age were found to vary among individual components. This might assist in explaining the inconsistency of mixture effects on ecosystem production along stand development. The higher ecosystem production in mixtures than expected from those of single species-dominated stands of a young age, was primarily attributed to the overyielding (positive mixture effects) of fine root production. With stand development, the negated effects of mixtures on ecosystem production were associated with decreased mixture effects on both aboveground tree and understorey vegetation production. The overyielding of fine root production, with no changes in entire ecosystem production in mixtures of mature and old stands, suggested a shift of production allocation among individual components. Together, these findings further suggested that the results generated from DPRs studies that focussed on specific production components (e.g., aboveground biomass and fine roots) (Zhang et al. 2012, Ma and Chen 2016) may not reflect entire

ecosystems, at least in forests, since various components may respond differently to species mixtures at a certain stand development stages.

Mixture effects on production partitioning along stand development

As expected, we found that mixtures significantly affect production allocation strategies between above- and belowground in natural forests. Importantly, we found that this effect changed along stand development; with mixtures allocating relatively more production to belowground in mature and old stands, in contrast to that in young stands. The increased production allocation to belowground that was observed in mixtures is in agreement with a previous finding (Nouvellon et al. 2012), which showed that the introduction of nitrogen-fixing tree species in fast-growing eucalypt plantations led to shifts in C allocation from above- to belowground for both species. However, there were no published studies for comparison that described age-related patterns of mixture effects on production allocation. Different responses of production fractions to species mixture and stand age among individual components were also observed in this study. This suggested that the relative amount of biomass production present in each individual component was not fixed, but may be affected by mixtures, as well as stand age. In young stands, the production fraction allocated to belowground even exceeded aboveground and mixtures slightly increased the production fraction allocated to fine roots at the expense of aboveground tree production, compared to single species-dominated stands. With stand development, the production fraction that was allocated to aboveground began to increase and exceed belowground and mixtures allocated an even higher production fraction to fine and coarse roots, at the expense of tree and understory vegetation growth, in contrast to single species-dominated stands.

The underlying mechanisms for the age-related dynamics of mixture effects on production allocation are complex and might be attributed to both physiological and ecological factors. Prior to canopy closure, since competition for light is not very strong, additional biomass is likely to be allocated belowground to acquire soil nutrients, which are limiting following fire (Hume et al. 2016). This confirmed what has previously been reported in the literature, which showed a shift in the allocation of NPP from aboveground coarse woody material, to fine roots when solar radiation was at its highest (Girardin et al. 2014). Another possible reason for the higher production fraction belowground in young stands, may be attributed to rapid vegetation colonization and increasing site occupancy, which are typical for boreal mixedwood forests during this stage of stand development (Greene et al. 1999). The slightly higher production fraction allocated to belowground in mixtures compared with single species-dominated stands may be related to the fact that the higher overall production of mixtures demands a higher population of roots to sequester the limited soil nutrients following forest fire. With stand development, soil N and P increased while light availability decreased, more production was allocated to aboveground to compete for light (Bloom et al. 1985). This is consistent with an earlier study, which demonstrated that an increasing stand age resulted in increased partitioning to aboveground wood production (Litton et al. 2007). Another possible explanation for increased aboveground production allocation is ontogenetic drift (Gedroc et al. 1996), which predicts the relative allocation of photosynthate is shifted from below- to aboveground (Coleman et al. 2004, Coyle and Coleman 2005, King et al. 2006, Peichl and Arain 2007). Compared to single-species dominant stands, mixtures allocated an even higher production fraction to fine and coarse roots at the expense of aboveground growth with stand development. The increased mixture effects of belowground

production allocation in mature and old stands may have been related the increased structural diversity (Brassard & Chen 2008) which helps reduce light stress by complementary use of light through vertical stratification (Yachi & Loreau 2007; Williams *et al.* 2017) and more production was shifted belowground (Casper *et al.* 2003).

Our results indicated that both biomass production and its partitioning were closely linked with soil nutrient availability, where PC1, which was contributed evenly by multiple key nutrients (e.g., N, P, K, Ca, Mg), had significant positive effects on the production of all components except understory vegetation and fine roots, while PC2, which was primarily contributed by N and Mg, only slightly impacted aboveground tree production. The positive association between aboveground production and soil nutrients indicated that soil nutrient availability mediated forest growth (Gower *et al.* 1996). The more pronounced influence of the first principle component of soil nutrients indicated synergistic effects of combined N, P, K, Ca, and Mg (Elser *et al.* 2007). Production partitioning appeared to be primarily driven by PC1, which increased production partitioning to aboveground components (e.g., stems and litterfall), while it decreased production partitioning to belowground (e.g., fine roots). Similarly, across all stand types and ages, the ANPP: BNPP ratio was found to decrease with PC1 (N, P, K, Ca, Mg); however, it was not affected by PC2 (N, Mg). These results were broadly consistent with the optimal partitioning theory (Bloom *et al.* 1985), as well as the findings of a number of recent papers, which showed that plants increased aboveground biomass allocation, with the increased availability of soil nutrients (Forrester *et al.* 2004, Litton *et al.* 2007). Since we find a significant increase of N, P, K, Ca, Mg along stand development, the increased production allocation to aboveground during stand development may attribute to the increased soil nutrient availability after fire (Hume *et al.* 2016).

5.6 Conclusion

The major limitation of published DPR studies in forest ecosystem is that none includes all components of productivity, preventing the detection of shifts in production allocation. This study presents the first comprehensive description of the entire ecosystem production and its partitioning among components and their response to species mixtures, stand age, and soil nutrient availability. We found that overyielding of entire ecosystem production only occurred in young stands and temporal change of mixture effects on production differ among individual components. Most remarkably, we reported a significant mixture effects on production allocation among individual component and mixtures allocated relatively more production to belowground than expected from single species-dominated stands. We also found a close linkage between production allocation and soil nutrient availability which may help explain the observed pattern of production allocation along stand age. The temporal change of mixture effects on both production and its allocation presented here is fundamental relevance to our understanding of diversity and productivity relationships. Our results will also contribute to terrestrial ecosystem models.

CHAPTER 6. GENERAL CONCLUSION

The major limitation of published DPR studies in forest ecosystems is that the vast majority of them have their focus set on aboveground components, based on snapshots of a single stand development stage, which prevents a comprehensive understanding of DPR. The findings of this dissertation extend our understanding of diversity effects on aboveground biomass production to belowground, as well as the entire forest ecosystem. Moreover, I provide a further elucidation of the mechanisms involved in observed temporal changes of mixture effects on fine root dynamics (e.g., production, turnover, and mortality). Finally, by encompassing all of the components of productivity, I detected shifts in production allocation among individual components along stand development in natural forest ecosystems. A summary of the key findings of this dissertation are as follows:

1. Through the synthesis of 48 published studies, I found that, on average, mixtures have 28.4% higher fine root biomass and 44.8% higher annual production than monocultures. Despite profound differences in environments among terrestrial ecosystems, belowground productivity responds similarly to variations in species richness. Furthermore, this study revealed shifts in diversity effects over time, in both forests and grasslands.
2. In my species diversity and fine root productivity study, I provided evidence of increasing diversity effects on fine root productivity with stand development. Moreover, I found that the increased diversity effects with stand development might have been the result of multiple processes. These include increasing horizontal and vertical soil volume filling, increasing forest floor depth/volume, and foraging limiting soil nutrients, all of which resulted in the more complete utilization of soil

space and nutrients, while benefiting from increased soil nutrient inputs and retention.

I note that these processes operate simultaneously.

3. In my species diversity and fine root turnover and mortality study, I found that, similar to biomass production, mixture effects on fine root turnover and mortality also increased with stand development. Moreover, I found that mixture effects shifted among soil layers with stand development and the increased mixture effects with stand development might have resulted from the increased competition intensity induced by the overyielding of fine root biomass production.
4. By synthesizing the production data of each individual components of forest ecosystems, I presented the first comprehensive description of production for the entire ecosystem, as well as production partitioning among components, and their responses to species mixtures, stand age, and soil nutrient availability. I found that overyielding of the entire ecosystem production occurred only in young stands, as various components respond differently to species mixtures at a certain stage in stand development. Most remarkably, I reported significant mixture effects on production partitioning among individual components, and mixtures partitioning relatively more production to belowground than was anticipated from single species-dominated stands. I also found an intimate linkage between biomass production and its allocation, and soil nutrient availability, which may assist with explaining the observed pattern of production allocation along with stand age.

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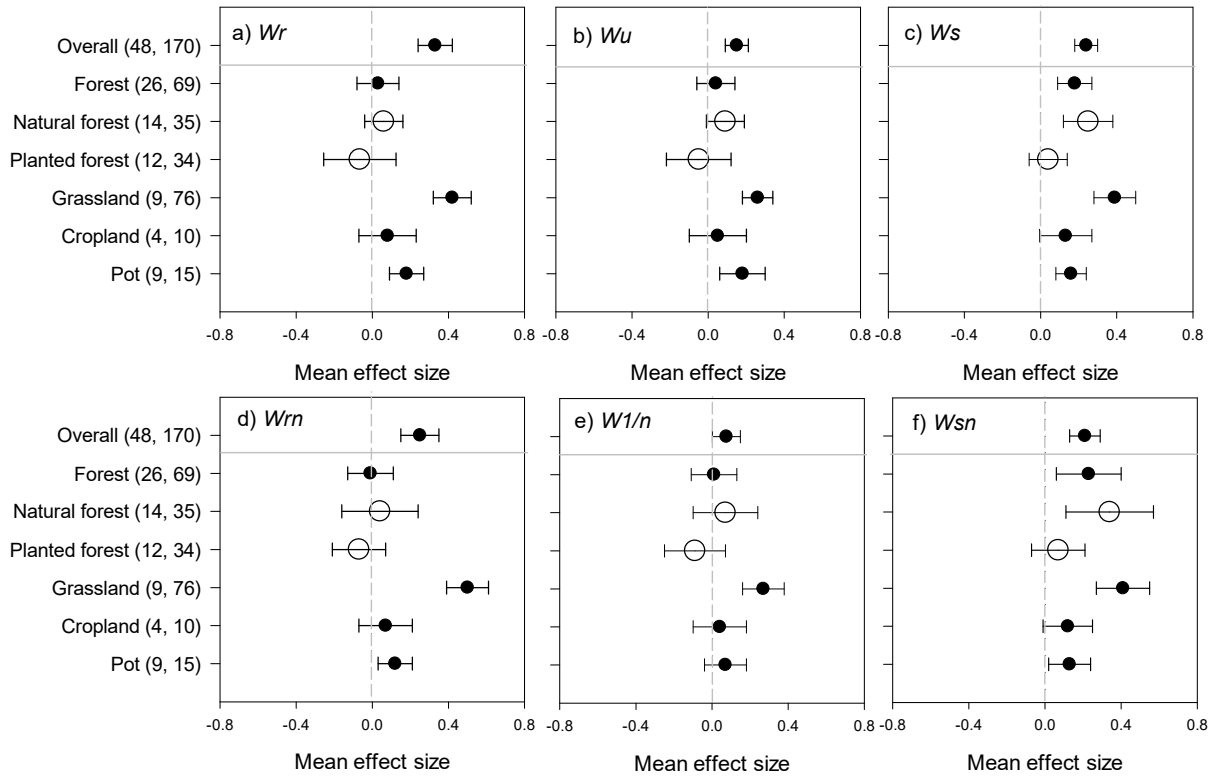
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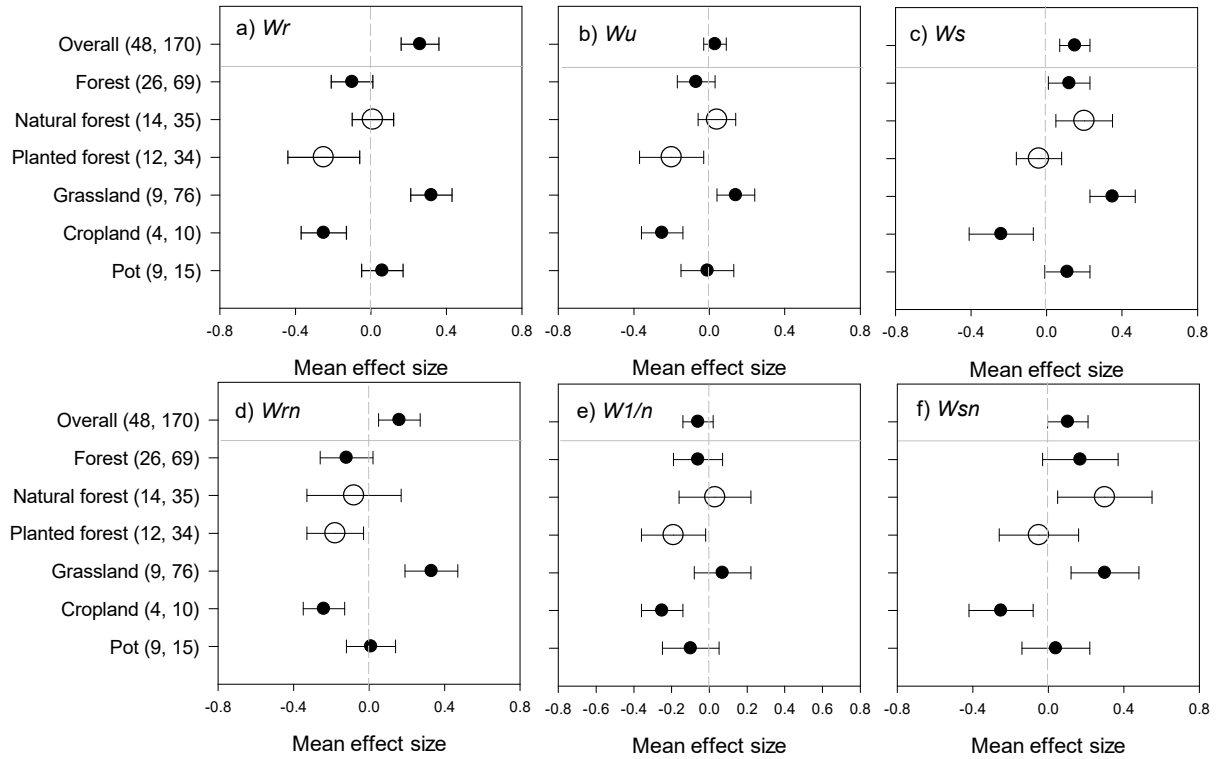
APPENDIX I. SUPPLEMENTAL INFORMATION FOR CHAPTER 2

Appendix 1. Data Source

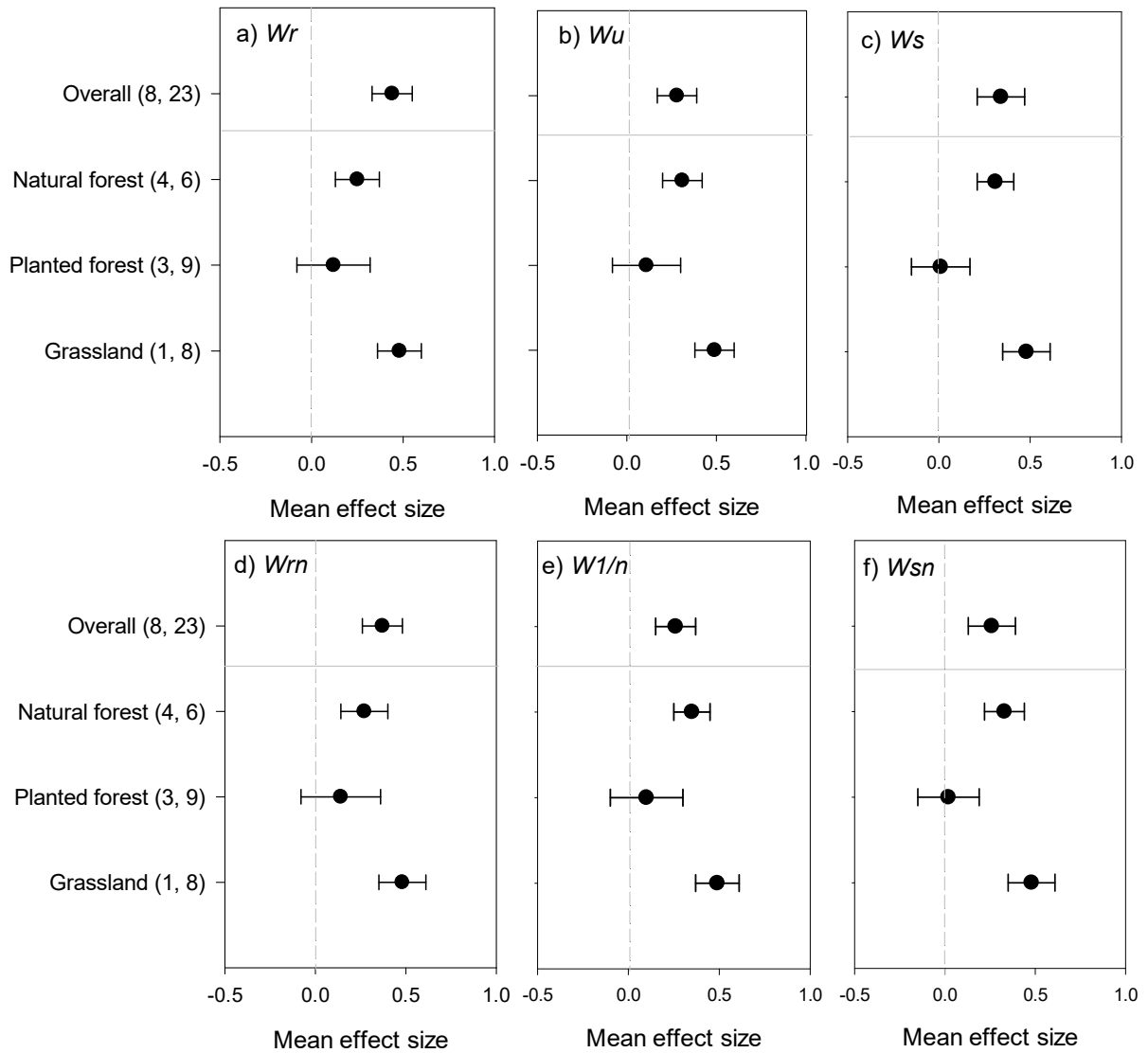
| Ecosystem type | Publication | Biomass | Production | |
|--------------------------------|-----------------------------------|-----------------------|-------------------|-----|
| Natural forest | Bolte and Villanueva (2006a) | Yes | | |
| | Bolte et al. (2013) | Yes | | |
| | Brassard et al. (2011b) | Yes | Yes | |
| | Brassard et al. (2013) | Yes | Yes | |
| | Jacob et al. (2013) | Yes | | |
| | Jacob et al. (2014) | | Yes | |
| | Hendriks and Bianchi (1995) | Yes | | |
| | Leuschner et al. (2001) | Yes | | |
| | Meinen et al. (2009b) | Yes | | |
| | Meinen et al. (2009a) | Yes | | |
| | Schmid (2002) | Yes | | |
| | Wang et al. (2002) | Yes | | |
| | Wang et al. (2014) | Yes | | |
| | Planted forest | Laclau et al. (2013) | Yes | |
| Domisch et al. (2014) | | Yes | Yes | |
| Lang'at et al. (2013) | | Yes | | |
| Lei et al. (2012a) | | Yes | Yes | |
| Bauhus et al. (2000) | | Yes | | |
| Brandtberg et al. (2000) | | Yes | | |
| Smith et al. (2013) | | Yes | | |
| Mckay and Malcolm (1988) | | Yes | | |
| Zhang et al. (2013) | | Yes | | |
| Liao et al. (1995) | | Yes | | |
| Wang et al. (2014) | | Yes | | |
| Grassland | | Mueller et al. (2013) | Yes | |
| | | Mommer et al. (2010) | Yes | |
| | | Reich et al. (2004) | Yes | Yes |
| | Ravenek et al. (2014) | Yes | | |
| | van Eekeren et al. (2010) | Yes | | |
| | Laossi et al. (2008) | Yes | | |
| | Niklaus et al. (2001) | Yes | | |
| | Skinner et al. (2006) | Yes | | |
| | Cropland | Fang et al. (2014) | Yes | |
| | | Xu et al. (2010) | Yes | |
| Xu et al. (2008) | | Yes | | |
| Corre-Hellou and Crozat (2005) | | Yes | | |
| Pot | Sanallah et al. (2011) | Yes | | |
| | Zhang et al. (2007) | Yes | | |
| | Beyer et al. (2013) | Yes | | |
| | Pausch et al. (2013) | Yes | | |
| | Dimitrakopoulos and Schmid (2004) | Yes | | |



Appendix 2a. Comparison of fine root biomass between mixtures and monocultures based on weighting functions. (a) Number of replications (w_r). (b) Unweighted approach (w_u). (c) Sampling variance (w_s). (d) The number of replications divided by the total number of observations from each study (w_{rn}). (e) By the inverse of number of observations from each study ($w_{1/n}$). (f) Sampling variance divided by the total number of observations of each study (w_{sn}). Results (bootstrapped mean and 95% confidence intervals of effect size) are shown for the entire data set (overall) and for forests, natural forests, planted forests, grasslands, croplands, and pot systems. The number of studies and observations of each ecosystem type is displayed in parentheses.



Appendix 2b. Comparison of fine root biomass between mixtures and most productive monocultures based on weighting functions. (a) Number of replications (w_r). (b) Unweighted approach (w_u). (c) Sampling variance (w_s). (d) The number of replications divided by the total number of observations from each study (w_{rn}). (e) By the inverse of number of observations from each study ($w_{1/n}$). (f) Sampling variance divided by the total number of observations of each study (w_{sn}). Results (bootstrapped mean and 95% confidence intervals of effect size) are shown for the entire data set (overall) and for forests, natural forests, planted forests, grasslands, croplands, and pot systems. The number of studies and observations of each ecosystem type is displayed in parentheses.



Appendix 3. Comparison of annual fine root production between mixtures and monocultures based on weighting functions. (a) Number of replications (w_r). (b) Unweighted approach (w_u). (c) Sampling variance (w_s). (d) The number of replications divided by the total number of observations from each study (w_{rn}). (e) By the inverse of number of observations from each study ($w_{1/n}$). (f) Sampling variance divided by the total number of observations of each study (w_{sn}). Results (bootstrapped mean and 95% confidence intervals of effect size) are shown for the entire data set (overall) and for forests, natural forests, planted forests, grasslands, croplands, and pot systems. The number of studies and observations of each ecosystem type is displayed in parentheses.

Appendix 4. Distribution of species richness in the mixtures across ecosystem types.

| Ecosystem | Mean | Min | Max |
|----------------|------|-----|-----|
| Overall | 4.7 | 2 | 31 |
| Forest | 3.1 | 2 | 5 |
| Natural forest | 3.8 | 2 | 5 |
| Planted forest | 2.6 | 2 | 5 |
| Grassland | 8.1 | 2 | 31 |
| Cropland | 2.0 | 2 | 2 |
| Pot | 3.2 | 2 | 6 |

Appendix 5. Effects of ecosystem type on the relationship between $\ln RR$ and richness. Cropland was not included since it only has one level of species richness.

| Source | d.f. | MS | <i>P</i> |
|--------------------------------|------|------|----------|
| $\ln(S)$ | 1 | 6.39 | <0.001 |
| Ecosystem type | 4 | 2.58 | <0.001 |
| $\ln(S) \times$ Ecosystem type | 3 | 0.13 | 0.12 |
| Error | 152 | 0.06 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS), and significance (*P*). $\ln(S)$ is natural log-transformed species richness.

APPENDIX II. SUPPLEMENTAL INFORMATION FOR CHAPTER 5

Table S5.1. Characteristics of 27 stands sampled in the boreal forest of Ontario, Canada. Stand types are single-species *P. banksiana* dominated (*Pb*), single-species *Populus tremuloides* dominated (*Pt*), and mixed *P. banksiana* and *P. tremuloides* (*Pb + Pt*). Each stand type was replicated three times. Values are means with 1 SE in parentheses.

| Characteristic | 8-years post fire | | | 34-years post fire | | | 85-year post fire | | |
|--|-------------------|-----------------|-----------------|--------------------|-----------------|-----------------|-------------------|-----------------|-----------------|
| | <i>Pb</i> | <i>Pt</i> | <i>Pb + Pt</i> | <i>Pb</i> | <i>Pt</i> | <i>Pb + Pt</i> | <i>Pb</i> | <i>Pt</i> | <i>Pb + Pt</i> |
| Stand basal area (m ² ha ⁻¹) | 1.55 (0.35) | 0.93 (0.33) | 1.39 (0.24) | 21.54 (1.07) | 25.56 (0.7) | 22.98 (1.57) | 37.13 (5.44) | 33.38 (6.29) | 33.82 (1.25) |
| Stand density (trees ha ⁻¹) | 5933 (1790) | 11533 (4173) | 9133 (1271) | 2091 (36.3) | 1733 (96.10) | 1233 (228.4) | 1458 (218) | 683 (54.6) | 1033 (172.2) |
| Tree species richness | 2.67 (0.33) | 2.67 (0.33) | 4.33 (0.33) | 2.00 (0.58) | 3.33 (0.88) | 2.67 (0.33) | 4.00 (0.58) | 2.00 (0) | 4.00 (0) |
| Tree species evenness | 0.13 (0.04) | 0.29 (0.07) | 0.57 (0.04) | 0.12 (0.11) | 0.23 (0.09) | 0.82 (0.11) | 0.34 (0.04) | 0.17 (0.05) | 0.72 (0.02) |
| Shannon's diversity index | 0.12 (0.03) | 0.30 (0.10) | 0.83 (0.09) | 0.14 (0.12) | 0.29 (0.13) | 0.81 (0.10) | 0.48 (0.11) | 0.12 (0.03) | 1.00 (0.03) |
| Tree species composition (% of stand basal area) | | | | | | | | | |
| | 97.62 | 2.96 | 67.28 | 96.31 | 2.06 | | 88.22 | | 42.51 |
| <i>Pinus banksiana</i> | (0.72) | (2.95) | (2.43) | (3.34) | (0.94) | 40.92 (8.74) | (3.00) | 0 | (11.62) |
| <i>Populus tremuloides</i> | 1.81 (1.62) | 91.86 (3.08) | 24.82 (2.11) | 4.62 (3.91) | 92.5 (3.54) | 52.12 (6.48) | 0 | 97.33 (0.92) | 44.06 (10.7) |
| <i>Picea mariana</i> | 0 | 0 | 0 | 0 | 0 | 10.43 (8.59) | 5.14 (1.56) | 0 | 6.03 (10.71) |
| <i>Salix</i> spp. | 0.81 (0.01) | 2.65 (0.56) | 0.85 (0.51) | 0 | 2.55 (1.89) | 0 | 0 | 0 | 0 |
| <i>Picea glauca</i> | 0 | 0 | 0 | 0 | 0 | 0 | 1.67 (0) | 0 | 0 |
| <i>Prunus pensylvanica</i> | 0 | 0.41 (0.41) | 1.56 (0.87) | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Betula papyrifera</i> | 0.63 (0.63) | 3.01 (3.01) | 6.27 (3.95) | 0.61 (0.61) | 6.18 (5.19) | 0 | 3.11 (0.36) | 2.67 (0.92) | 7.40 (2.95) |

Table S5.2. Effects of stand age on mixture effects for individual components and total ecosystem production.

| Source | d.f. | ATP | | UP | | LP | | CRP | | FRP | | TEP | |
|-----------|------|------|----------|------|----------|-------|----------|------|----------|------|----------|------|----------|
| | | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> |
| Stand age | 2 | 0.10 | 0.18 | 0.22 | 0.10 | 0.002 | 0.99 | 0.09 | 0.77 | 0.11 | 0.43 | 0.06 | 0.24 |
| Error | 6 | 0.04 | | 0.07 | | 0.167 | | 0.09 | | 0.09 | | 0.03 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS) and significance (*P*).

Table S5.3. Effects of stand age on mixture effects for individual components and total belowground production fraction.

| Source | d.f. | ATPF | | UPF | | LPF | | CRPF | | FRPF | | BLF | |
|-----------|------|------|----------|------|----------|------|----------|------|----------|------|----------|------|----------|
| | | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> | MS | <i>P</i> |
| Stand age | 2 | 0.04 | 0.19 | 0.06 | 0.41 | 0.05 | 0.51 | 0.03 | 0.59 | 0.27 | 0.06 | 0.20 | 0.10 |
| Error | 6 | 0.02 | | 0.05 | | 0.07 | | 0.05 | | 0.05 | | 0.02 | |

Listed are source of variation, degree of freedom (d.f.), mean sum of squares (MS) and significance (*P*).

Table S5.4. Effects of soil nutrients on biomass production of individual components and total ecosystem.

| Attribute | ATP | | UP | | LP | | CRP | | FRP | | TEP | |
|-----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|
| | Slope | <i>P</i> | Slope | <i>P</i> | Slope | <i>P</i> | Slope | <i>P</i> | Slope | <i>P</i> | Slope | <i>P</i> |
| Intercept | 3.40 | <0.001 | 0.37 | <0.001 | 1.87 | <0.001 | 0.28 | <0.001 | 2.83 | <0.001 | 8.75 | <0.001 |
| PC1 | 0.55 | <0.05 | 0.01 | 0.64 | 0.36 | <0.001 | 0.06 | <0.001 | -0.17 | 0.17 | 0.82 | <0.05 |
| PC2 | 0.94 | <0.05 | -0.01 | 0.82 | 0.19 | <0.001 | 0.04 | 0.37 | 0.18 | 0.56 | 1.34 | 0.06 |

Table S5.5. Effects of soil nutrients on production fraction of individual components and total belowground.

| Attribute | ATPF | | UPF | | LPF | | CRPF | | FRPF | | BLF | |
|-----------|-------|----------|--------|----------|--------|----------|---------|----------|-------|----------|-------|----------|
| | Slope | <i>P</i> | Slope | <i>P</i> | Slope | <i>P</i> | Slope | <i>P</i> | Slope | <i>P</i> | Slope | <i>P</i> |
| Intercept | 0.34 | <0.001 | 0.05 | <0.001 | 0.20 | <0.001 | 0.031 | <0.001 | 0.37 | <0.001 | 0.41 | <0.001 |
| PC1 | 0.04 | <0.05 | -0.005 | 0.08 | 0.027 | <0.001 | 0.005 | <0.05 | -0.07 | <0.001 | -0.06 | <0.001 |
| PC2 | 0.05 | 0.14 | -0.007 | 0.32 | -0.003 | 0.81 | -0.0002 | 0.96 | -0.04 | 0.28 | -0.04 | 0.28 |

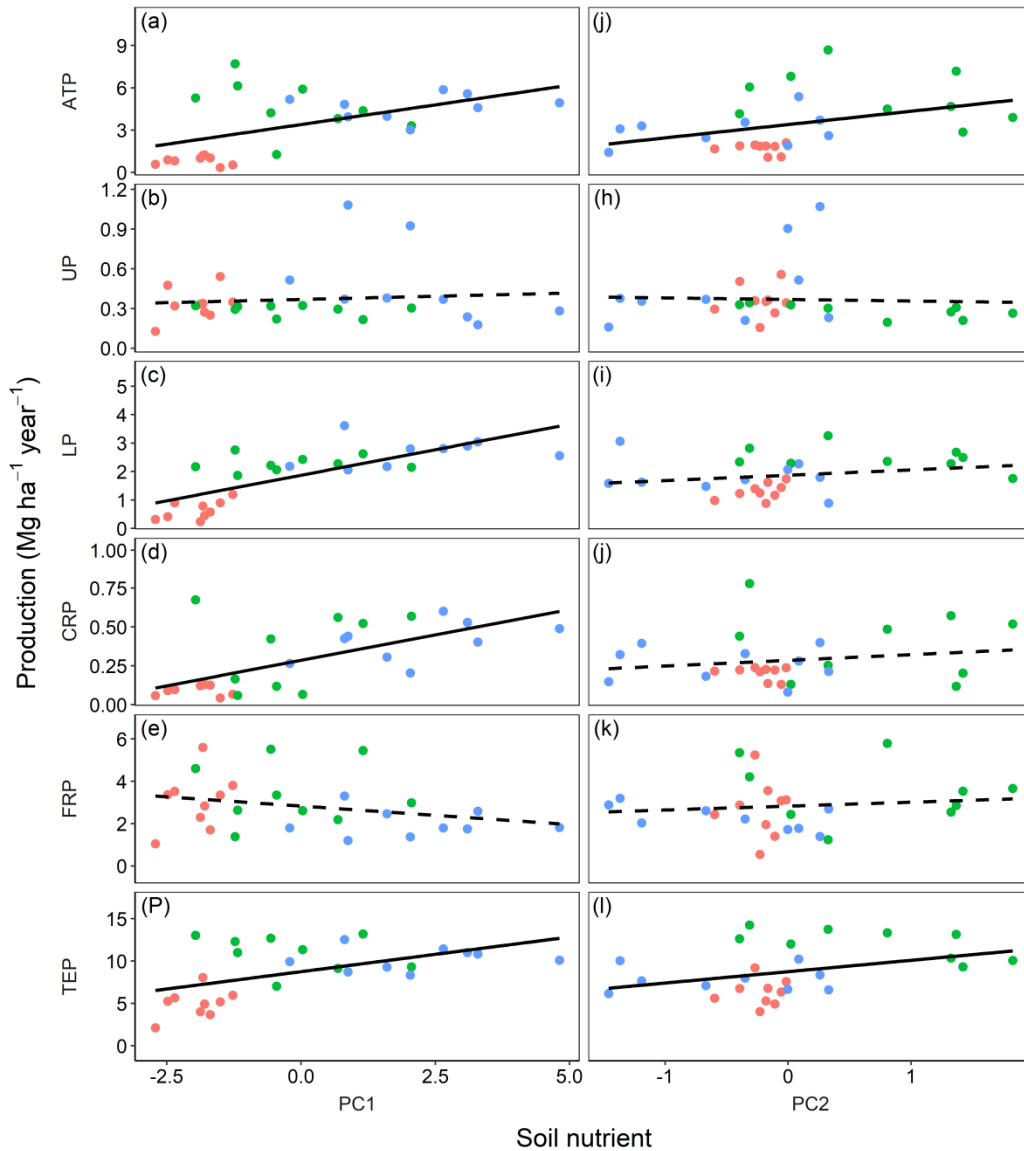


Figure S5.1. Partial regression plots show the biomass production of individual components as well as the entire ecosystem in relation to the first two principle components of soil nutrients (PC1 and PC2). Colours indicate stand age classes: red, 8 years; green, 34 years; blue, 85 years. Within each panel, the solid line represents a significant relationship through all three stand types and ages ($n = 27$), while the dashed lines represent an insignificant relationship.