Mycorrhizas and root traits of the riparian tree species *Populus fremontii*

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Forest Biology and Management

Department of Renewable Resources

University of Alberta

Abstract

In hot and arid environments, trees may be particularly carbon-limited. In addition to supporting their own growth and activities, trees also lose carbon to associate with mycorrhizal fungi. Most trees form associations with either ecto- or arbuscular mycorrhizal fungi, and these fungi can have distinct effects on quantities of carbon released from fine roots and other root traits. Forests in arid regions are not uncommon, however trees in these areas must budget carbon as a limited resource, suggesting variation in carbon allocation to roots and mycorrhizal fungi could be important for overall tree fitness. *Populus fremontii* occurs in the southwest United States, a region that has experienced more droughts with climate change. Increasing aridity throughout its range means *P. fremontii's* future will be progressively more carbon-limited. Moreover, unlike most other plant species, this species simultaneously associates with both ecto- and arbuscular mycorrhizal fungi, thus making it possible to decouple the effects of mycorrhizal type from tree species identity.

I tested the influence of mycorrhizal type on root traits, including fine root carbon flux, using provenances of *P. fremontii* known to vary in dominance of the two mycorrhizal types. In both a field study and greenhouse experiment, I confirmed not only that *P. fremontii* forms ecto- and arbuscular mycorrhizas simultaneously, but depending on provenance and environment, individuals also preferentially associate with one mycorrhizal type over the other. While I did not measure carbon allocation from *P. fremontii* to each mycorrhizal type, high colonization rates of both mycorrhizal types suggests carbon is transferred from trees to support these fungi. Mycorrhizal type, however, did not affect fine root carbon flux in either experiment, and overall, I measured extremely low fine root carbon-flux rates. In addition, root traits were variable and disconnected from mycorrhizal type, revealing highly context-dependent patterns. These results

suggest that *P. fremontii* minimizes exudation of carbon across a range of environments, and the distinct effects of mycorrhizal type on root traits are nuanced. Compared with other tree species in wetter environments, *P. fremontii* is a conservative species in regard to fine root flux, with no indication that mycorrhizal fungi moderate this belowground process. As aridity increases in regions with historically wetter environments, *P. fremontii* could signal how other tree species under increasing carbon-limitation, might modify root traits including fine-root carbon flux and how this could influence belowground processes such as carbon cycling.

Preface

The research for this thesis forms part of an international research collaboration, led by professor Dr. Catherine Gehring at Northern Arizona University, with professor Dr. Justine Karst as the lead collaborator at the University of Alberta. All chapters included in this thesis are my original work. No data from this thesis has been published prior to thesis defense and publication.

Acknowledgments

First, I would like to give a huge thanks to two people who, without them, this work would not have been possible, Justine Karst and Kitty Gehring. This project was initially confronted with significant challenges, and due to the unwavering support and patience from both Justine and Kitty, this project is complete. Justine's mentorship and guidance through this process pushed me to think creatively and strive for improvement at every step. Justine provided this opportunity for me and continued to have incredible flexibility from start to finish, for which I will always be grateful. Kitty welcomed me into her lab and provided invaluable resources allowing me to pursue my research in Arizona. Her deep knowledge of cottonwood ecology was critical to developing this work and my understanding of ecological principles. Thank you, Justine and Kitty. Your enthusiasm for science is contagious!

I would also like to give a massive shout-out to all my fellow lab mates both in Edmonton and Arizona. I will always be thankful for all the vital feedback, brainstorming sessions, and happy hour beers, which profoundly improved my experimental design, alternative explanations, and overall mental health while working on these chapters. Additionally, I would like to thank Pak Chow for his expertise, technical guidance, and organization. Pak's help was a critical component in completing both data chapters.

Last but not least, I would like to thank my family and friends. Their love and support have never failed me over the last couple of years. I could not have done this without you. Thank you!

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Chapter 1: General introduction

Roots and their associated microbiomes, specifically the mycorrhizal fungi with which they interact, influence belowground ecosystem processes including carbon (C) cycling and sequestration (Clemmensen et al., 2013). The mycorrhizal symbiosis is a relationship mediated by resource exchange in which the fungus acquires soil resource needed by its plant partner, and, in turn, the plant provides the fungus with fixed C (Smith & Read, 2008). Prevalent in many plant species, mycorrhizae divide into functional types exhibiting distinct characteristics including the physical mechanism of interaction with host root, associated C cost, and resource acquisition strategy regulating which key nutrients are exchanged with the host (Smith & Read, 2008). The two most common groups of fungi forming mycorrhizas are arbuscular (AM) and ectomycorrhizal (EM) fungi, and are distinct in their traits and interactions with host roots. For example, EM fungi are generally considered a more C expensive partner (Smith & Read, 2008), and could 'pull' more photosynthetic C from the tree into the soil. One consequence of differences in C costs could be the variation of hyphal extensions into the soil between ecto- and arbuscular mycorrhizal fungi. Ectomycorrhizal fungal hyphae are quite extensive in soils (Högberg & Högberg, 2002), while AM hyphae are less extensive but have fast turnover rates quickly cycling C through its hyphal networks (Staddon et al., 2003). Furthermore, EM fungal hyphae can be more recalcitrant with thick, pigmented, and septate walls, in relation to AM fungi and as a result, EM fungal tissue generally has a long residence time in soils (Langley & Hungate, 2003). Hyphal extensions are just one example of trait variation between mycorrhizal types that could impact decomposition rates and further C cycling in soils through increased residence time and more recalcitrant necromass of EM fungi.

How the dominance of EM trees relative to AM trees influences C and nutrient cycling in forests has been described by the “Mycorrhizal Associated Nutrient Economy” (Phillips et al., 2013). In this conceptual model, forests dominated by either EM or AM trees, are characterized by organic and inorganic nutrient economies, respectively. The difference in nutrient economies emerge primarily through differences in decomposition rates of litter varying in quality, ultimately leading to greater fractions of nutrient pools in organic or inorganic forms. AM forests have high quality litter, which is rapidly decomposed mostly by free-living saprotrophs in soils increasing inorganic nutrient pools of which, AM fungi can access and trade with their host tree. Conversely, EM forests have low quality litter, which is degraded by EM fungi, leading to increased sequestration of carbon and nitrogen. Moreover, dominant mycorrhizal type and their ability to influence ecosystem processes like C cycling and sequestration has been used to model global C stocks (Soudzilovskaia et al., 2019).

Though a potentially powerful predictor of nutrient economies in forests, mycorrhizal type is often conflated with tree species identity and the environments where these relationships occur. For example, environmental factors such as soil pH or mean annual temperature, can explain the distribution of mycorrhizal type in plant species (Bueno et al., 2017), potentially confounding mycorrhizal-mediated ecosystem processes with abiotic conditions. Furthermore, the mechanism underlying the effect of mycorrhizal type on ecosystem processes is unresolved. For instance, AM and EM dominated temperate forests showed mixed decomposition rates over time, leaving mycorrhizal type effect on root decay rates unclear (Sun et al., 2018). Tree species, which associate with fungi from multiple mycorrhizal types simultaneously, otherwise known as 'dual-colonized', are an ideal model system to investigate the influence of mycorrhizal type on nutrient and C cycling. As tree species identity can be held constant, the distinct influence of AM

and EM fungi in C cycling and sequestration may be isolated in dual-colonized tree species. Through molecular and microscopy techniques, combined with field and greenhouse observations, many tree genera have been determined to be 'dual-colonizing', namely able to associate with both AM and EM fungi simultaneously (Teste et al., 2020). These tree species are ideal for disentangling the influence of host-species identity, local environment, and mycorrhizal type on belowground C cycling. However, few studies have used 'dual-colonizing' species to understand root morphological, chemical, and physiological trait variation as it relates to mycorrhizal type.

As a novel approach to understanding the influence of mycorrhizal type on various root traits linked to C cycling, I used the dual-colonizing species *Populus fremontii* (Fremont Cottonwood) in a set of complementary experiments. My overall study objectives were to: 1) confirm this species associated with two mycorrhizal types simultaneously, 2) test if dominate mycorrhizal type would change over environmental conditions and 3) understand how dominate mycorrhizal type would influence root traits, such as fine root C flux. In my first data chapter, I selected provenances of *P. fremontii* known to differ in their extent of EM and AM fungal colonization allowing me to understand the degree to which 'dominant' mycorrhizal type impacts commonly measured root traits. In my second data chapter, I attempt to directly manipulate EM and AM colonization to test the effect of mycorrhizal type on root traits across different provenances. As I use similar root trait measurements across both studies, I look for repeated patterns within this tree species across environmental conditions.

Chapter 2: Carbon flux and other fine root traits display inconsistent patterns when associated with mycorrhizal fungal types

2.1 Introduction

The release of labile carbon (C) from roots into the surrounding soil, otherwise known as exudation, has gained interest in belowground ecological research, and is considered a driving trait in the root economic spectrum (Guyonnet et al., 2018; L. Sun et al., 2021; Wen et al., 2022). Plants can lose 5–21% of photo-fixed C in the form of organic compounds into the rhizosphere (Haichar et al., 2014), and these plant-derived root inputs are more efficient in the formation of soil organic C than aboveground litter deposition (Sokol et al., 2019). Although a seemingly small percentage of fixed C, exudation is a continuous seeping of C from plant to rhizosphere stimulating soil biota, stabilizing soil C, and representing a form of constant communication between plants and their soil environment (Bais et al., 2006; Jones et al., 2004). Due to their vast geographical range, large C pools, and influence on ecosystem processes, exudation from fine roots of trees is a prominent focus in exudation studies. As a dynamic process, exudation has been shown to vary across tree species (Sun et al., 2021), substrate type (Meier et al., 2020), soil moisture content (Jakoby et al., 2020), and increased atmospheric CO_2 concentration (Phillips et al., 2009). Additionally, within and across studies, exudation rates have varied by tree mycorrhizal type (ecto- or arbuscular mycorrhizal) (Liese et al., 2018; Sun et al., 2021; Yin et al., 2014). In addition to coinciding with fine root exudation rate of C, tree mycorrhizal type has also correlated with distinct nutrient economies affecting C cycling and storage (Phillips et al., 2013). Typical exudation values from the field are very small and can range from 6.2 – 66.7 μg C cm^{-2} root day^{-1} to 0.27 – 2.42 μg C cm^{-1} root day^{-1} (Jakoby et al., 2020; Phillips et al., 2008).

Two general patterns have emerged from *in-situ* observations; 1) however small, all trees exude some quantity of C into soils, and 2) mycorrhizal type influences belowground C flux rates of plants. From these past studies, two knowledge gaps have merged.

First, most studies are conducted in temperate or tropical forests with moderate to high precipitation (709–1330 mm year $^{-1}$) and mild mean annual temperatures (7.1–11.6 °C), aside from one field experiment located in the Mediterranean (509 mm year^{-1} of precipitation; see (Jakoby et al., 2020)). Furthermore, trees within these forests might not be particularly C-limited as they have relatively highwater availability and moderate temperatures, meaning trees can actively photosynthesize with little consequence of excessive water loss and leaf senescence. Hence, C allocation might be less restricted to primary pools such as growth and respiration permitting transfer of C belowground as exudation. This emphasis on moderate environments in which exudation is currently understood begs the question, will trees growing in more C-limited, marginal environments exude similar amounts of C as trees in wetter, cooler environments? As climate change increases temperature and decreases precipitation in some areas of the world, a shift in focus to marginal environments where forests are currently experiencing these conditions will be helpful for a more robust understanding of exudation processes.

Second, though mycorrhizal type (ecto- (EM) and arbuscular mycorrhizas (AM)) has been associated with the rate of C flux from roots, it is conflated with species identity because often a tree species is a single mycorrhizal type. Previous work established preliminary hypotheses on how EM and AM fungi influence C flow from roots to soil (Churchland & Grayston, 2014). As fundamental players in C cycling, fungi use C from host plants for their own biomass and metabolic activities depending on mycorrhizal type, life-stage, and environmental conditions (Smith & Read, 2008). For example, AM hyphae can take recently photo-assimilated

C and quickly transfer it to soil microbes before passive root exudation can occur, making the fungal partner a possible C-sink for the plant, and altering the C transfer from root to hyphal exudation (Kaiser et al., 2015). Moreover, Liese et al. (2018) investigated the influence of watering treatments and found that EM trees have higher exudation rates under drought conditions compared to their AM counterparts, meaning EM fungi can alter exudation dynamics of their host, possibly at a larger magnitude than AM dominated species. Though there is direct evidence that root C exudation differs between EM and AM fungi, mycorrhizal type is often conflated with tree species identity and the environments where these relationships occur. Moreover, the mechanism underlying the effect of mycorrhizal type on exudation processes is unresolved. Trees that reside in marginal environments and interact with fungi of multiple mycorrhizal types simultaneously will be critical subjects to understand C flux in more adverse environments.

Single host species which associate with two mycorrhizal types simultaneously, i.e., 'dual-colonized' hosts, are an ideal model system to investigate the influence of mycorrhizal type on ecosystem processes such as fine root C flux. As AM and EM fungi may control the flow of C from plant roots to soil, it is critical to understand the individual role of each fungal type in the C cycle (Finlay, 2008; Jansa et al., 2013). However, the influence of mycorrhizal type on ecosystem processes has often used basal area of tree species assigned as either AM or EM as a proxy for abundance of these two types of fungi in soils (Brzostek et al., 2015; Phillips et al., 2013; Soudzilovskaia et al., 2019). The characterization of AM vs. EM trees often confounds tree phylogeny with mycorrhizal mechanisms affecting belowground ecosystem processes. Trees assigned to one mycorrhizal type typically reside within the same family and there is less phylogenetic overlap across mycorrhizal types than within, e.g., it is common for studies to

assign Fagaceae as EM and Aceraceae as AM (Craig et al., 2018; Liese et al., 2018; Sun et al., 2021). Furthermore, by investigating 'dual-colonized' species, I can disentangle mycorrhizal type from host identity, which to my knowledge has not been done in exudation studies and is essential to evaluate mycorrhizal partners and their role in belowground C cycles. Dual-colonized plants are not uncommon , and new techniques for identifying these plants have identified 89 genera that associate with both ecto- and arbuscular mycorrhizas (Teste et al., 2020).

One genus of dual-colonized plants is *Populus*, which can simultaneously associate with AM and EM fungi (Karst et al., 2021; Meinhardt & Gehring, 2012). In the case of *Populus fremontii* (Fremont cottonwood), the extent of mycorrhizal colonization by AM versus EM fungi of an individual tree is a consequence of many abiotic and biotic factors (Gehring et al., 2006; Gehring & Whitham, 2002; Hultine et al., 2020). As a foundation species, *P. fremontii* is vital to riparian corridors acting as oases or "ribbons of green" in the arid ecosystem's expanse across the U.S. Southwest (Grady et al., 2011; Webb et al., 2007). Throughout its native range, *P. fremontii* experiences highly variable and stressful abiotic conditions, creating distinct genetic provenances with potentially high phenotypic plasticity from heritable traits (Cooper et al., 2019), including associations with mycorrhizal fungi (Gehring et al., 2006). Gehring et al. (2006) and Hultine et al. (2020) observed a genotypic basis for EM and AM colonization of cottonwoods from two extreme environments. Thus, provenances could differ in the proportion of AM and EM colonization resulting in a natural gradient of mycorrhizal type occurring in more C-limited marginal environments than previous studies.

Leveraging a natural gradient in mycorrhizal type of *P. fremontii*, I sought to investigate how fine root C exudation relates to the dominance of AM and EM fungal colonization. There

were three objectives for this study; 1) confirm dual-colonized status in *P. fremontii* and investigate colonization rates across a climatic gradient, 2) determine whether fine-root C exudation differs between EM and AM dominated *P. fremontii*, and 3) map root economic traits to understand this species' belowground adaptations to the marginal environment in which it is found. To address this latter objective, I measured morphological, chemical, and physiological traits to understand relationships among root characteristics and mycorrhizal associations.

2.2 Methods

2.2.1 Field sites selection

The Southwest Experimental Garden Array (SEGA) includes several common gardens of *Populus fremontii* (S. Watson), established along an elevational gradient of approximately 2,000 meters across six degrees of latitude (Cooper et al., 2019; Hultine et al., 2020). These gardens encompass varying environmental conditions that provenances of *P. fremontii* naturally experience. I selected the two most climatically extreme gardens within SEGA for my study: (1) the low elevation garden (LEG), located outside of Yuma, Arizona along the U.S.-Mexico border, has a mean annual temperature (MAT) of 23.6 °C and annual precipitation (MAP) of 72 mm, and (2) the high elevation garden (HEG), located at Canyonlands Research Center, Utah, has a MAT of 11.6 °C and MAP of 221 mm (Fig. 2. 1). Average daily temperature during the time of the study at the LEG was 25.7 °C with 36.6% relative humidity and the HEG was 21.3 °C with 12.5% relative humidity. The LEG watering regime consisted of flood irrigation once a month, while the HEG was drip irrigated three times a week for three hours.

2.2.2 Cottonwood provenances by mycorrhizal type

In 2014, each garden was reciprocally transplanted with individuals from 16 natural provenances located across the Colorado Plateau. Critical to my study, provenances planted across the gardens are known to vary in their mycorrhizal associations based on provenance elevation (Hultine et al., 2020). Hultine et al. (2020) found that, when planted in a mid-elevation garden, one low elevation provenance, native to the Sonoran Desert, had a 6:1 ratio of AM: EM colonization and that a high elevation provenance, from southern Utah, had approximately the inverse ratio. To indirectly manipulate mycorrhizal type of individual trees, I chose focal

provenances from high (>1,000 m) and low elevations (<1,000 m) across the *P. fremontii's* native distribution from two of its three ecoregions (Hultine et al., 2020). Provenances were initially selected from an early spring mortality survey. At LEG, I selected two provenances from each elevation class, and at HEG, owing to mortality of low-elevation provenances, I was restricted to sample only three high elevation provenances (Table 2. 1 & Fig. 2. 1).

2.2.3 Tree selection and physiological traits measured

Seven to ten trees were selected for each of the chosen provenances; in each garden these trees were at least 1 m apart but within 5 m distance from one another. All trees were randomly selected with at least 1 m from plot boundaries to reduce edge effects. Trees were seven years old at the time of sampling, showed no obvious signs of disease, and had healthy crown foliage when selected. Diameter at breast height (DBH; cm) and height (m) of each tree was measured. To approximate potential differences in photosynthetic capacity among trees, three leaves were sampled at approximate heights of 1.8 to 2 m from each tree and ImageJ (https://imagej.nih.gov/ij/) was used to scan each for area, following by drying and weighing to calculate specific leaf area (SLA; $cm^2\ g^{-1}$). Prior to their removal, stomatal conductance (g_s; mol $H_2O\ m^{-2}s^{-1}$) of each of the three leaves was measured with a Li-Cor 600 (Li-Cor Biosciences 2020) across one day at three different time periods (9 AM, 12 PM and 3 PM) for all trees.

2.2.4 Measurement of root exudation

Fine root exudation of C was measured *in-situ* during "peak" performance when leaves were thoroughly flushed, which is defined in this study as more than 60 days after average first bud flush of provenances for each garden (Cooper et al., 2019). Provenances in LEG were measured from the 2nd to 7th of May 2021, and during the week of the 7th to 14th of June 2021 for

those in HEG. At each sampling period, exudates were collected over one week using the culture-based system described by Phillips et al. (2008). Roots were selected within 0.5 m of focal trees and carefully excavated to avoid damaging roots or mycorrhizas. Fine root segments (<2 mm in diameter) from the terminal ends, approximately 15–20 cm in length, were cleaned by carefully rinsing the root segment in a C-free nutrient solution (0.5 mM NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.2 mM $MgSO_4$, and 0.3 mM $CaCl_2$). Each cleaned root terminal was placed in a glass syringe (Poulten & Graf Ltd, Barking, UK) and filled with 1 mm acid-washed glass beads (Cole-Parmer, Vernon Hills, USA) along with an infusion tube, and the entire cuvette system was then sealed and covered to avoid contamination and reduce light and extreme heat. Sterile C-free nutrient solution was pumped into the glass cuvette through the infusion tube to keep roots moist and to avoid nutrient stress. After a 48-hour incubation period, cuvettes were flushed five times with approximately 50 mL of sterile C-free nutrient solution to rinse roots and left to rest for 24 hours. After the second incubation period, the sterile C-free nutrient solution was flushed through the cuvettes, immediately filtered with sterile 0.22 µm syringe filter (Thermo-Fisher Scientific, Waltham, USA), and collected in sterile containers. A total of three controls were established per day of root excavation per garden using the same procedure described above without a root segment and placed next to a sample with roots. Each exudation sample was kept cold and stored at -20 °C until run through a Total Organic Carbon (TOC) analyzer for non-particulate organic C (NPOC) at the METAL Lab Facility (Arizona State University, Tempe, AZ). Fine root (0.1–1 mm diameter) length was used to standardize carbon flux rates, because these roots are assumed to be responsible for most of the C absorption and release. Positive C flux (µg C cm^{-1} day^{-1}) values indicate exudation, while negative values

indicate C absorption within the cuvette system when compared to the control after adjusting for the detection level (0.5 mg L^{-1}).

2.2.5 Root measurements

After exudates were collected, roots in each cuvette were cut from the tree and transported back to Northern Arizona University on ice packs and stored at 4 °C for no more than 12 hours. Roots were scanned and root diameter, total root length, root area and volume, and number of root tips was analyzed by WINRHIZO PRO 2013 software (Regents Instruments Inc., Quebec City, QC, Canada). Once scanned, all roots were stored at -20 °C until scored for EM colonization, and subsequently cleared and stained for AM colonization. To measure root colonization by EM fungi, roots were scored under a dissecting microscope using the grid intersect method (Brundrett et al., 1996). Ectomycorrhizal morphotypes have been identified for these provenances (Markovchick in prep., 2022) and served as the basis for identification of fungal presence on root tips. Afterwards, to identify structures of AM fungi, roots were cleared in 10% KOH solution at room temperature for 10–14 days depending on the melanization of roots, then stained with a 0.5% Trypan blue solution. Roots were then scored for presence of hyphae, vesicles and arbuscules according to the gridline intersect method under 20× magnification (Brundrett et al., 1996).

In addition to the roots retrieved from each cuvette, two additional root samples were collected by tracing back to the same trees sampled for exudation. These extra root samples had an average root diameter of 0.38 mm and were oven dried for one hour at 100 °C and then 72 hours at 65 °C and placed in a freezer until weighed and measured for total non-structural carbohydrates (NSC). Total fine root starch and sugar concentration were quantified by

peroxidase-glucose oxidase and acid assays respectively as per Chow & Landhäusser, 2004. Values were measured as mg g^{-1} of dry weight (DW).

2.2.6 Statistical Analysis

All statistical analysis was conducted in R (R Team, 2022) with the following packages: plyr (Wickham, 2011), tidyr (Wickham and Henry, 2019), vegan (Oksanen et al., 2022), ape (Paradis and Schliep, 2019), ecodist (Goslee and Urban, 2007), and corrplot (Wei and Simko, 2021). All data were inspected visually through residual plots, and normality assumptions were met. Outliers were inspected for influential observations by Cook's distance, however when further identified, all outliers were from the same provenance and hence not removed.

Due to tree mortality that prevented sampling of the same provenances in both gardens, gardens were analyzed separately and only compared qualitatively. To address the first objective, i.e., to confirm the dual-mycorrhizal status of cottonwoods, within each provenancea two-sample t-test was run to compare mean percent colonization between mycorrhizal types. To address the second objective, an analysis of variance was run to test the correlation between mycorrhizal colonization and fine root C flux rates.

Lastly, to address the third objective three and explore commonly measured root economic traits of *P. fremontii* at each garden, a principal component analysis (PCA) was run on belowground traits including: specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 g^{-1}), fine root C flux (C flux; μg C cm^{-2} day^{-1}), percent mycorrhizal colonization (AM and EM), root tissue density (RTD; g cm^{-3}), and starch and sugar NSC concentrations (mg g^{-1} DW)). A Pearson's correlation matrix was then used to examine significant relationships from each PCA.

Additionally, for each garden, an analysis of variance was run to test for difference among provenances for each measured variable. Within each garden, there were few differences among provenances in root traits and thus, provenances were combined. Within each provenance, a two-sample t-test was run to compare mean fine root starch and sugar concentrations. Differences among NSC types, across and within provenances, expanded our understanding of C allocation and storage strategies within fine roots.

2.3 Results

Belowground Traits

2.3.1 Mycorrhizal colonization gradient

In both gardens, roots of all trees were colonized by both AM and EM fungi (Fig. 2.2). At the LEG, the ratio of mean AM:EM was approximately 3:2, in contrast to 1:3 at the HEG (Fig. 2.2, (LEG: $t(29) = 4.7$, $p < 0.001$; HEG: $t(29) = -16$, $p < 0.0001$)). At the LEG, EM colonization ranged between 3 and 41% ($\bar{x} = 19 \pm 1.6$ SE). Higher EM colonization at the LEG was associated with provenances from higher elevations, however this pattern was only significantly higher for one provenance, L3 (27% EM colonization, $p = 0.05$, Fig. 2.3; Table S2.1), compared to the other provenances. Arbuscular mycorrhizal colonization ranged from 11 to 44% ($\bar{x} = 29 \pm 2.0$ SE), however there were no significant differences among provenances at the LEG (Fig. 2.3; Table S2.1). At the HEG, colonization across the provenances ranged between 34 and 73% for EM ($\bar{x} = 59.6 \pm 1.77$ SE) and between 6 and 43% for AM ($\bar{x} = 20.8 \pm 1.44$ SE). Colonization did not differ among provenances at the HEG (Fig. 2.4; Table S2.2).

2.3.2 Fine root carbon flux

Between the gardens, fine root C flux ranged from -1.2 to 0.67 ($\bar{x} = -0.20 \pm 0.0019$ SE) ($\mu g\ C\ cm^{-1}\ day^{-1}$)(Fig. 2.5 & 2.6). At the LEG, fine-root C flux values did not differ among provenances (Table S2.3) and ranged from -1.2 to 0.24 ($\bar{x} = -0.20 \pm 0.059$). The HEG fine-root C flux values ranged from -0.98 to 0.27 ($\bar{x} = -0.20 \pm 0.060$) with no significant differences among provenances (Table S2.4). On average, at each garden there was no exudation but absorption of C (Fig. 2.5 & 2.6). Carbon flux was not significantly correlated with either type of mycorrhizal colonization at either garden (LEG; AM - ($F(1, 28) = 2.6$, $p = 0.12$), EM - ($F(1, 28) = 0.22$, $p =$

0.65), Fig. 2.7 & HEG; AM - ($F(1, 27) = 4.2$, $p = 0.051$), EM - ($F(1, 26) = 2.0$, $p = 0.17$), Fig. 2.8).

2.3.3 Root non-structural carbohydrates

At the LEG, provenances did not differ in either NSC type; mean total sugar concentration was 18.5% (±1.6 SE) and mean total starch concentration was 14.9% (±3.1 SE) (Fig. 2.9; $t(40) = -1.0$, $p = 0.30$, Table S2.5). At the HEG, total sugar and starch concentrations did not differ among provenances (Fig 2.10; Table S2.6), however sugar concentrations were 38 times higher than starch ($\bar{x}$ = 32.6 and 0.839 mg g^{-1} DW, respectively; Fig. 2.10; $t(27) = -13$, $p < 0.0001$).

2.3.4 Combined root traits

When viewed in multi-trait space, different patterns emerged at each garden (Fig. 2.11 & 2.13). The most consistent relationships were a positive correlation between SRA and SRL, and a negative correlation between RTD and SRA at both gardens (Fig. 2.12 & 2.14). Beyond that, each garden exhibited distinct patterns among all other measured traits (Fig. 2.11 & 2.13). Total root length, fine root surface area (SA) and total SA did not differ among provenances within each garden (Table S2.7 & S2.8).

At the LEG, there were negative correlations between SRL and EM, and SRA and AM colonization. Root starch and sugar concentrations were positively correlated as well as starch and RTD (Fig. 2.12). Root tissue density negatively correlated with SRL and SRA (Fig. 2.12). The only trait that did not correlate with others when combined in multivariate space was fine root C flux (Fig. 2.11 & 2.12).

At the HEG, EM colonization negatively correlated with RTD and no other variable, while AM colonization was positively correlated with root sugar concentrations and negatively correlated with starch concentrations (Fig. 2.13). Root tissue density was negatively correlated with SRA and no other variable (Fig. 2.13). Fine root C flux did not correlate with other traits in this multivariate space (Fig. 2.13 & 2.14).

Aboveground Traits

Size and physiology of trees differed between the two gardens. Trees were short with small DBH's, but high leaf stomatal conductance at the LEG, while trees at the HEG exhibited the opposite pattern with tall trees with large DBHs and low stomatal conductance (Fig. 2.15 & 2.16). At the LEG, all traits differed among provenances (Fig. 2.15; Table S2.11, $p < 0.001$) with the largest trees found in the L1 provenance and decreases in height with increases in provenance elevation of origin. Stomatal conductance exhibited the opposite trend, increasing as provenance elevation of origin increased. Notably, L3 individuals were much smaller in height and DBH, and had higher stomatal conductance relative to the sympatric provenance (Fig. 2.15). Height, DBH, and stomatal conductance did not differ among provenances at the HEG. (Fig. 2.16; Table S12).

2.4 Discussion

2.4.1 Populus fremontii is simultaneously colonized by ecto- and arbuscular mycorrhizal fungi

Through direct observations of mycorrhizal structures in every root system, I confirmed that *Populus fremontii* associates with both AM and EM fungi simultaneously, across a range of abiotic conditions and provenances. As important model systems to disentangle plant-fungal interactions, without confounding host effects, the verification of dual mycorrhizal associations will be important for future studies (Teste et al., 2020). While specific genera, such as *Populus*, are recognized as dual-colonized, without field data, this "dual" status is not certain and could incorrectly estimate the functionality of a species in relation to specific ecosystem processes associated with each mycorrhizal type. In fact, *in-situ* observations of *Populus tremuloides* have demonstrated that, in its northern range, sexually mature trees were primarily colonized by EM fungi and often no AM structures were found within its roots (Karst et al., 2021). Simultaneous colonization of species is a dynamic trait, and mycorrhizal switching, or the shift in dominant mycorrhizal type, can be a consequence of host/fungal life stage, water, or nutrient availability, and is important to note while measuring colonization at one spatial or temporal point (Teste et al., 2020).

While AM and EM were found in every tree sampled, the two common gardens exhibited different dominant mycorrhizal types. The larger ratio of AM: EM at the LEG and EM: AM at the HEG, is consistent with previous work where ecotype drives mycorrhizal colonization rates (Hultine et al., 2020). Provenances sampled from each garden can be split into two established ecotypes, High-Plateau and Sonoran Desert (Hultine et al., 2020, Table 2.1). For example, provenances L1-L4 are sourced from a wide area, all of which is encompassed in the Sonoran Desert ecotype. In addition, the location of the LEG is also with the Sonoran Desert ecotype

range. The same is true for the provenances measured at the HEG and the garden itself, however for the High-Plateau ecotype. Meaning the provenances included in my study were growing within similar climatic conditions and genetic groupings where they were sourced. Interestingly, while the U.S. Southwest has experienced more frequent and severe drought events since 2000 (USDM 2022), EM colonization dominated at the HEG. This is contrary to similar common garden experiments, where watering treatments increased EM and decreased AM colonization (Gehring et al., 2006). My results show that despite low watering and increased aridity at the HEG, EM was dominant in fine roots. Additionally, the abundant water supply at the LEG could have shifted the dominant mycorrhizal type of provenances from AM to EM, however, AM remained the dominant mycorrhizal type within this garden. Consistent mycorrhizal type dominance despite shifts in abiotic conditions might suggest a greater genetic control over this root traits and environmental conditions played a smaller role.

2.4.2 Low rates of fine root carbon flux

The generally positive values of fine root C flux of tree species in the field have been scaled up to understand annual C flux rates of measured tree species (Meier et al., 2020; Phillips et al., 2008). However, I show that not all tree species exude substantial amounts of C into the soil environment. In stressful environments where C is limited by drought and high temperatures, as reflected by measured low stomatal conductance rates, measurable quantities of C may not be exuded into the rhizosphere. While previous studies have suggested that tree roots generally release small amounts of C, they have not been conducted in hot, dry environments where C acquisition may be limited, such as in the habitat of *P. fremontii* in the United States Southwest. Overall C flux rates were negative ($\bar{x}$ = -0.21 μg C cm^{-1} day^{-1}), meaning the roots exuded so little C it was below the detection limit and that some labile C from the nutrient solution was absorbed

by fungi, bacteria, or another free-living organism within the cuvette. The study of *in-situ* exudation is relatively new and only a handful of studies using this cuvette system have been published, all in areas with considerably higher annual precipitation rates and more moderate temperatures than the system I studied (Jakoby et al., 2020; Liese et al., 2018; Meier et al., 2020; Phillips et al., 2008; L. Sun et al., 2017; Yin et al., 2014). The results of my study highlight the need for future work across a variety of abiotic conditions and tree species to improve the breadth of our understanding of fine root C flux.

Populus fremontii habitat is along riparian corridors in arid ecosystems with extreme precipitation and temperature fluctuations (Leffler & Evans, 1999). Moreover, my reported stomatal conductance values were lower than previously reported rates for this species (Grady et al., 2013). These environments exemplify unique ecosystems that are unlike locations used for other exudation studies, representing C-limited environments, where hot and dry temperatures inhibit C uptake in leaves (Aparecido et al., 2020). I am confident in the C flux values, as this study encompassed two independent field trials, one test trial in October of 2020 (data not shown), and greenhouse measurements (see Chapter 3), and ranges of C flux remained consistent. Nevertheless, exudation collection in the field is notoriously difficult and current methods should be carefully compared across studies as detection limits, control values, and units have not been standardized and are either not reported or differ greatly among studies (Oburger & Jones, 2018).

This study is the first of its kind to investigate fine root C flux of a dual-colonizing species in an attempt to disentangle mycorrhizal type and host identity, which are often conflated. In the current study, fine root C flux was not related to colonization levels of AM or EM fungi, suggesting that tree species may play a larger role than mycorrhizal type on fine root

C flux. The C flux rates reveal that even provenances which differed in their AM: EM ratios do not show distinct C flux patterns in the same garden environment. New conceptual frameworks incorporating root exudation into root economic traits correlates high exudation rates with fast growing species and infertile soils, and not mycorrhizal association (Wen et al., 2022). Current theory of mycorrhizal type influencing a swath of belowground ecosystem processes though mechanisms like exudation could overestimate the functional consequences of associations with AM versus EM fungi. Although I do not quantitatively compare garden sites, both gardens have similar rates and fall within the range of 2.0 to -2.0 μg C cm^{-1} day^{-1}.

There are multiple pathways by which C leaves roots; exudation from roots and exudation from fungal associates such as mycorrhizal fungi. While roots can release labile C in a variety of forms, hyphae of both mycorrhizal types also exude C containing compounds (Drigo et al., 2010; Kaiser et al., 2015; Y. P. Sun et al., 1999; van Hees et al., 2006). Analogous to root exudation, the release of C containing compounds from hyphae can weather, decay, and access nutrients in the soil environment, which are not available to plants (Gadd, 2007). The methods by which I collected root exudation in the field, effectively eliminated mycorrhizal hyphal networks that extend past the rhizosphere and into the bulk soil. By excluding these hyphae, C flux could have been underestimated, as I can only measure what hyphae remain in the cuvette. It could be the case that C is drawn preferentially out of the roots by mycorrhizal fungal species and then exuded by the fungus, not the root. Additionally, because the cuvettes are sterile environments, the remaining hyphae, which can actively absorb recently released C (Y. P. Sun et al., 1999), could have absorbed whatever small quantities of C were previously exuded. This leaves two additional pathways in which C may have been moved in this system: 1) release of C by the fungus through hyphal extensions and not the root, and 2) C which has been recently released

that is then reabsorbed by fungal hyphae. In this study, I investigated the root pathway of C from the root and into the soil environment, and I cannot rule out the possibility that these *in-situ* methods eliminate fungal exudation pathways.

2.4.3 High intraspecific variation of root economic traits

I measured various root characteristics including biotic (mycorrhizal interactions), physiological (exudation), morphological (root diameter and SRL), and architectural (branching intensity) traits that have broad impacts on ecosystem processes (Bardgett et al., 2014). Traditionally taxa have been mapped onto these traits representing a continuum of conservative and competitive species whose belowground traits are reflective of their fast and slow growth strategies (Reich, 2014). However, these traits are more nuanced than can be captured on a two-dimensional scale and multidimensional projections propose two gradients that can appropriately represent these combined root traits: 1) the conservation gradient which describes traditional fast and slow growth strategies and the trait trade-offs between each, and 2) the collaboration gradient which emphasizes mycorrhizal association as an "out-sourcing" strategy for nutrient and water acquisition (Bergmann et al., 2020). For example, species such as *Cornus officinalis* and *Ginkgo biloba* have relatively high mycorrhizal colonization rates placing them on the "outsourcing" side of the collaboration gradient, but they diverge on the conservation gradient with higher C investments being made by the slower growing *Cornus officinalis* separating out subtle differences of root economic traits between taxa (Bergmann et al., 2020). My results do not consistently match these established gradients, and when viewed in multivariate space, I show that *P. fremontii* is not constant in its root morphological traits, suggesting highly plastic root traits across the two measured eco-types.

In a more C-limiting environment such as the HEG, increased SRL (lower C investment per unit length) is positively correlated with percentage colonization for both AM and EM fungi, suggesting highly efficient roots that also utilize mycorrhizal fungi. However, relative to its counterpart, the LEG, with less C-limited conditions, trees exhibit root traits which are consistent with an "outsourcing" strategy where fine, thin roots have less mycorrhizal colonization than more thick coarse roots. One pattern I find is a high correlation between SRL and SRA, as roots become finer, they also become longer, indicating that these roots become more efficient in both length and surface area in exploiting the soil environment. In addition, RTD was negatively correlated with SRA at both garden sites, but not SRL, which is inconsistent with the collaboration and conservation gradients, where RTD and SRL are mapped to each respective gradient, conservation, and collaboration, and are uncorrelated to each other (Wen et al., 2022). However, it is important to note that established root economic gradients were modeled from various taxa and mapping traits within a species is critical to understand plastic traits in changing conditions. *Populus fremontii* shows highly plastic aboveground traits including bud set which, depending on the source provenance, were adaptive traits which increased survival or mal-adaptive traits increasing mortality (Cooper et al., 2019). Our sampling period was 7 years since these trees have been planted and mortality, specifically at the HEG, only allowed me to sample from surviving provenances at each garden from which there was no overlap. To understand the adaptability of belowground root traits within different gardens, overlapping provenances should be chosen to decouple environmental and genetic drivers.

While not typically included in the root economic spectrum traits, NSC concentrations are a critical component to C allocation strategies (Körner, 2003), although most theoretical frameworks focus on aboveground NSC concentrations (Sala et al., 2012). Measurement of

starch and sugar concentrations in roots could improve belowground trait modeling. Non-structural carbohydrate concentration differed greatly among ecotypes and was correlated with distinct traits at each garden. Starch was positively correlated with RTD at the LEG, as a large and heavy compound, high starch concentrations and increased RTD could be a consequence of starch storage. Conversely, at the HEG, only AM colonization was correlated with NSC concentrations and exhibited inverse patterns between starch and sugar. Higher sugar concentrations were positively correlated with increased AM colonization, while starch displayed the opposite pattern. Few studies have compared NSC concentrations with mycorrhizal colonization and conclusions remain inconsistent with increased (Wu & Xia, 2006), mixed (Graham et al., 1997), and no association (Jongen et al., 1996) of NSC concentrations with mycorrhizal inoculation. Overall lower concentrations of NSC at the HEG is most likely a reflection of reduced C uptake aboveground from limited water availability due to previous drought events. Considering the large trees at the HEG, the C which is being fixed within the leaves may be prioritized to the primary pools including aboveground biomass while transfer to belowground storage is not prioritized. As the LEG had ample water supply throughout the year, C-uptake through photosynthesis, as indicated by increased stomatal conductance rates, was higher at the LEG than the HEG. Increased storage of NSC at the low elevation garden could reflect increased C-uptake.

2.4.4 Conclusion

This study establishes that within two extreme environments, *P. fremontii* interacts with two mycorrhizal types simultaneously and dominant mycorrhizal type varies between sites. The dominance of a mycorrhizal type across provenances despite the vastly different abiotic conditions between gardens, provides evidence for genetic influence over fungal partner

association. However, it is still uncertain the degree to which the fungus influences belowground process in this tree species as multivariate analysis shows inconsistent relationships between mycorrhizal colonization and root economic traits. Moreover, the lack of correlation between mycorrhizal type and fine root C flux, might indicate that host species identity plays a larger part in root-derived C flux. Extremely low fine root C flux values in this study indicates that not all species actively release large amounts of C belowground. However, due to field sampling restrictions, this methodology does not isolate hyphal exudation which could be an alternative hypothesis to our negative C flux values of this heavily colonized dually associating tree species. Finally, intraspecific variation between root economic traits could indicate a variety of trait adaptations to the marginal environments that *P. fremontii* encounters throughout its geographical range.

Figures

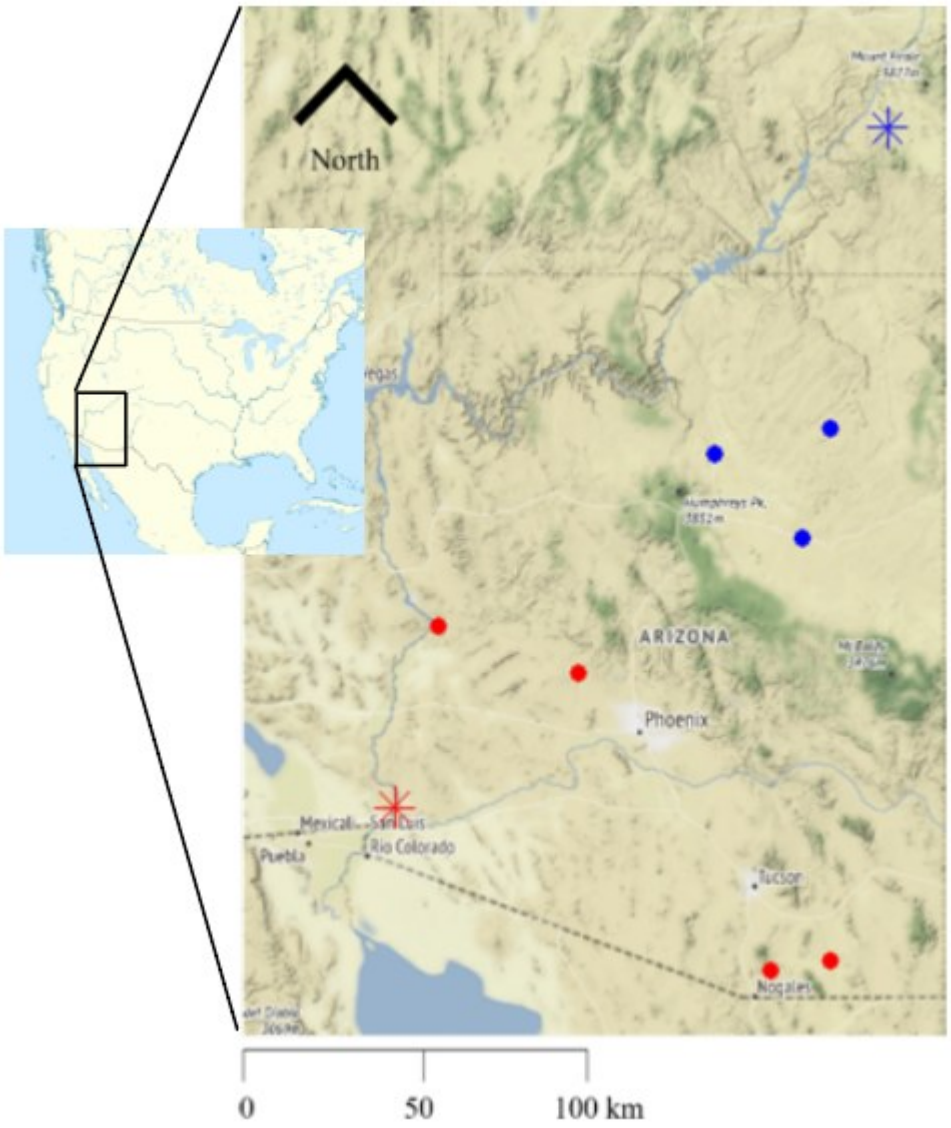

Figure 2. 1. Location of Southwest Experimental Garden Array common garden sites (stars) and provenances (dots) of cuttings planted into each garden, Low Elevation Garden (LEG) – red star and red dots for L1, L2, L3, and L4, High Elevation Garden (HEG) – blue star and blue dots for H1, H2, and H3 (see Table 2.1 for provenances codes).

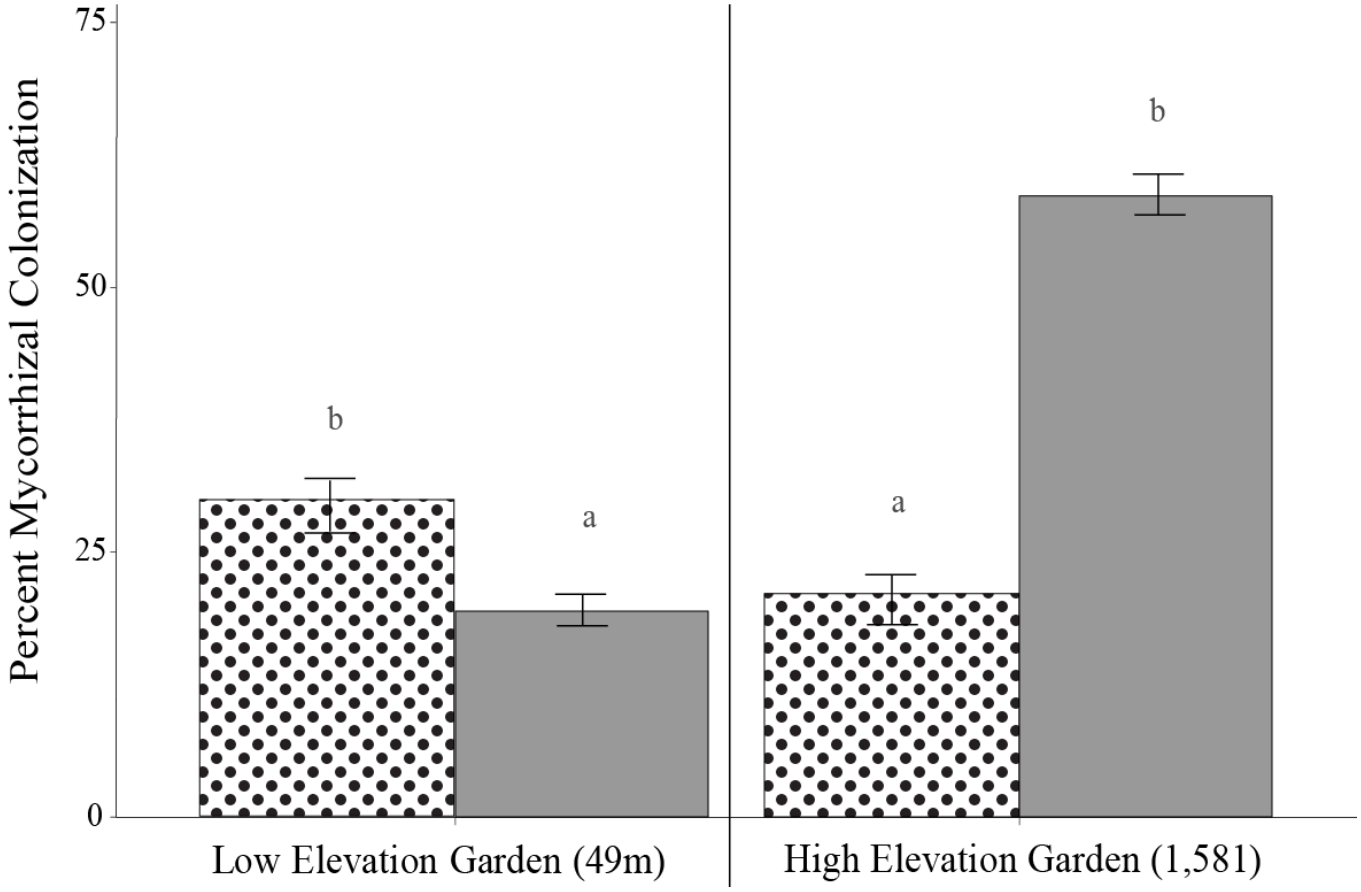

Figure 2. 2. Mean (± 1SE) ectomycorrhizal (solid) and arbuscular mycorrhizal colonization (dotted) of *Populus fremontii* roots at a) Low Elevation and b) High Elevation gardens. Significant differences in colonization between mycorrhizal types are indicated by different letters.

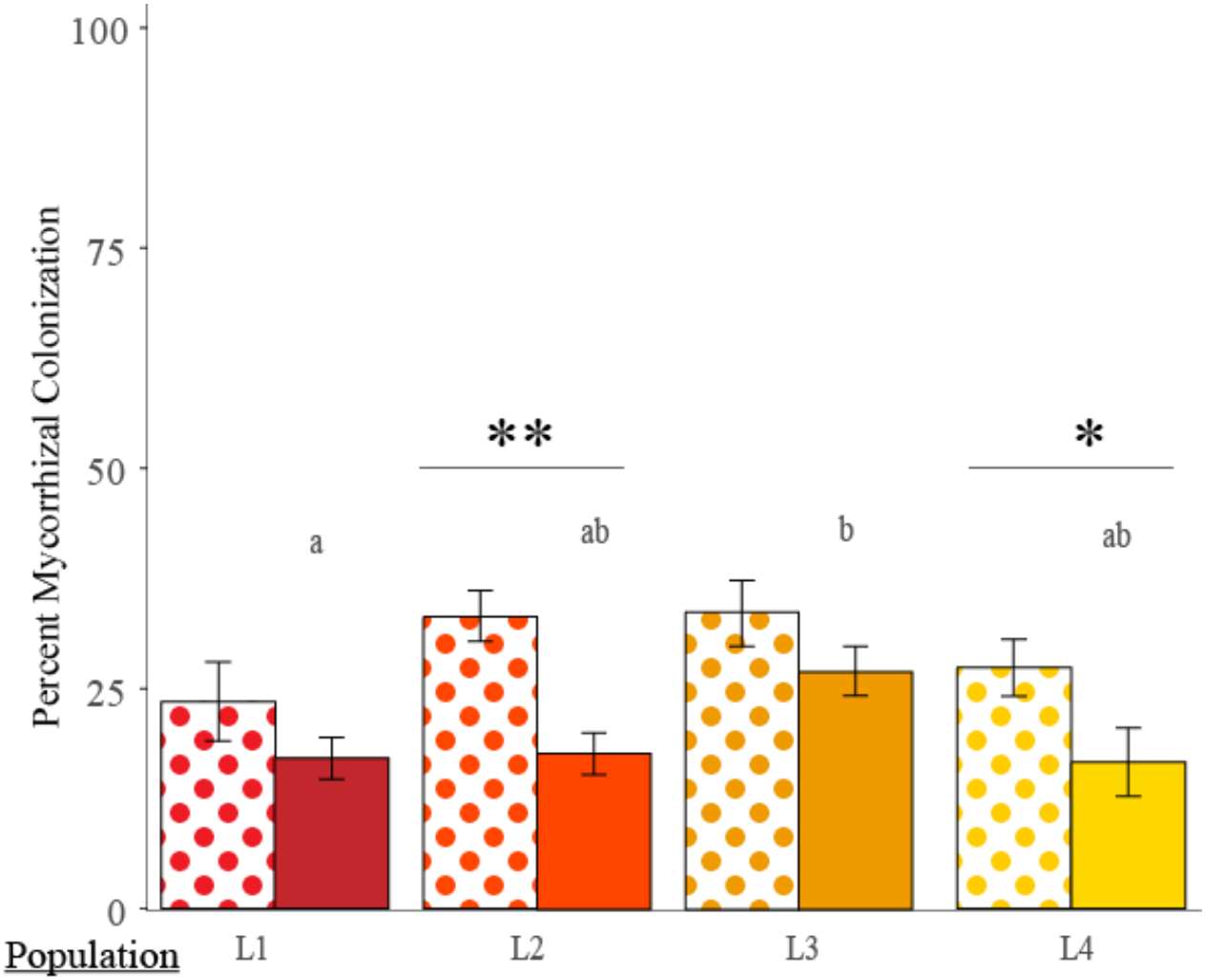

Figure 2. 3. Mean (± 1SE) mycorrhizal colonization of *Populus fremontii* by arbuscular (AM (dotted)) and ectomycorrhizal (EM (solid)) fungi of the four provenances sampled in the Low Elevation Garden. Letters indicate significant differences among provenances for EM colonization (p- value < 0.05). Lines indicate differences of mycorrhizal type within provenances (p-values are as follows: * <0.05, ** < 0.01).

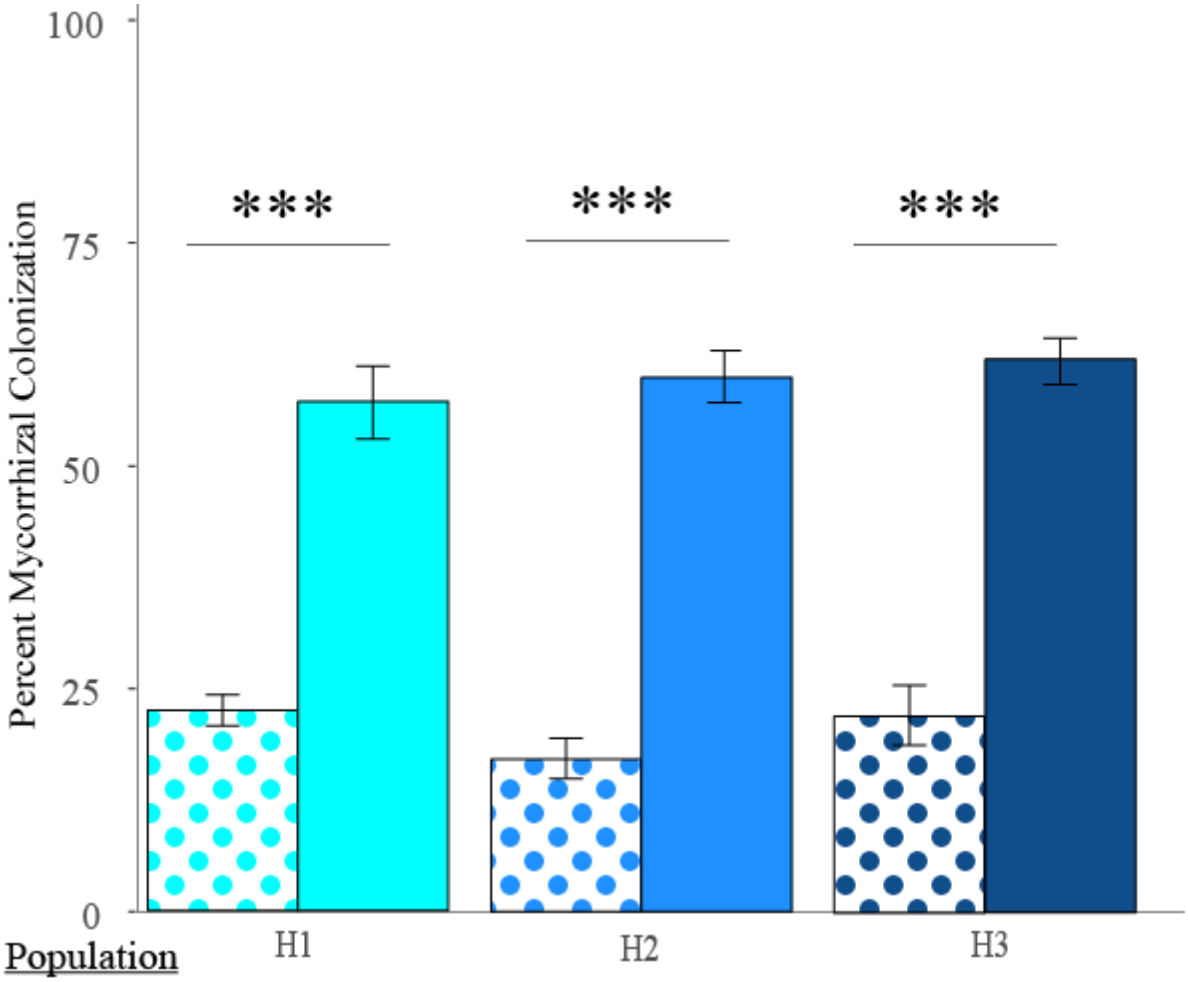

Figure 2. 4. Mean (± 1SE) mycorrhizal colonization of *Populus fremontii* by arbuscular (dotted) and ectomycorrhizal (solid) fungi of the three provenances sampled at the High Elevation Garden. There were no significant differences among provenances. Lines indicate differences of mycorrhizal type within provenances (p-values are as follows: *** < 0.001).

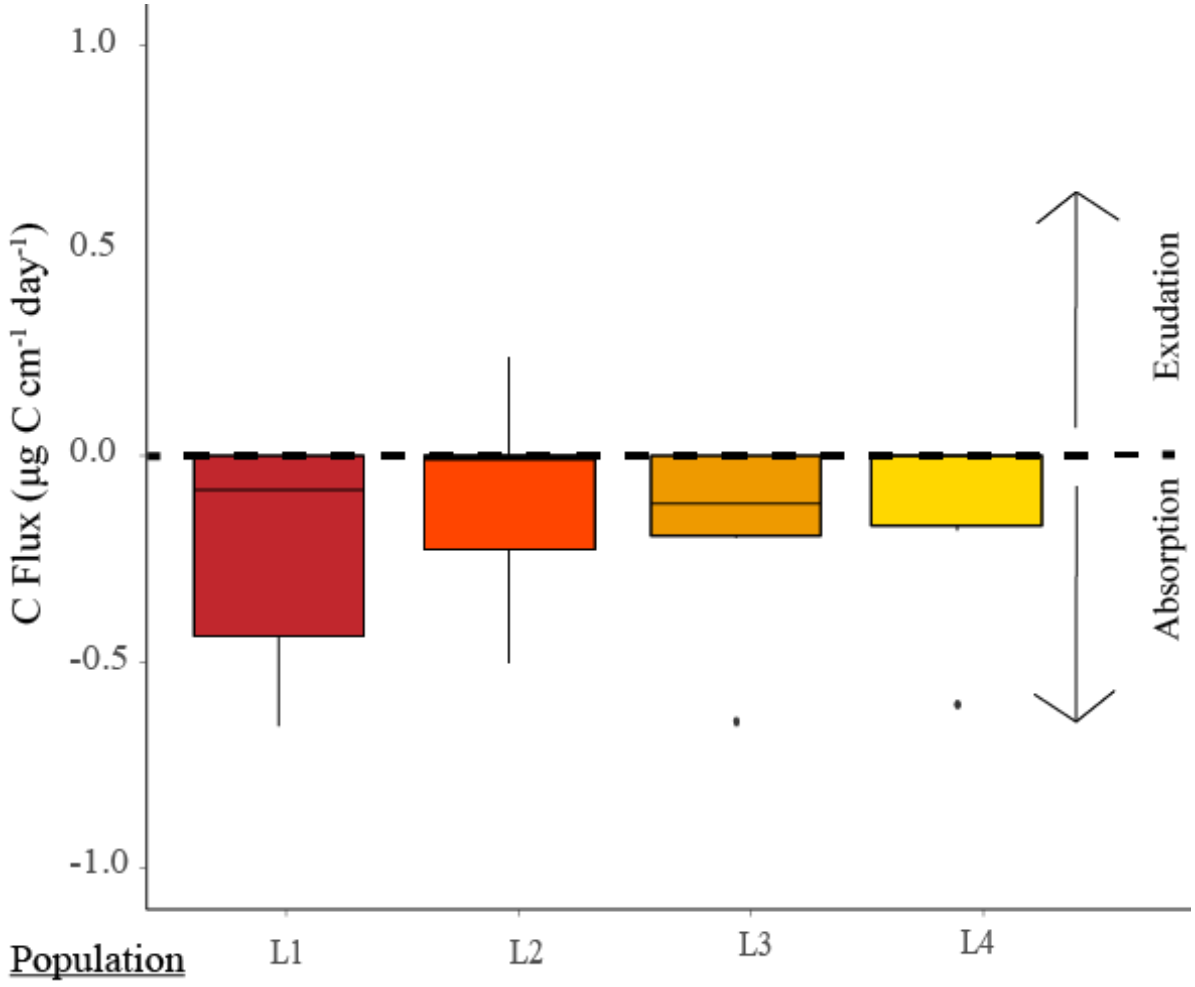

Figure 2. 5. Box and whisker plot indicating median, 25th and 75th percentiles as boxes and the 10th and 90th percentiles as error bars of fine root C flux (µg C cm^{-1} day^{-1}) of *Populus fremontii* measured at the Low Elevation Garden.

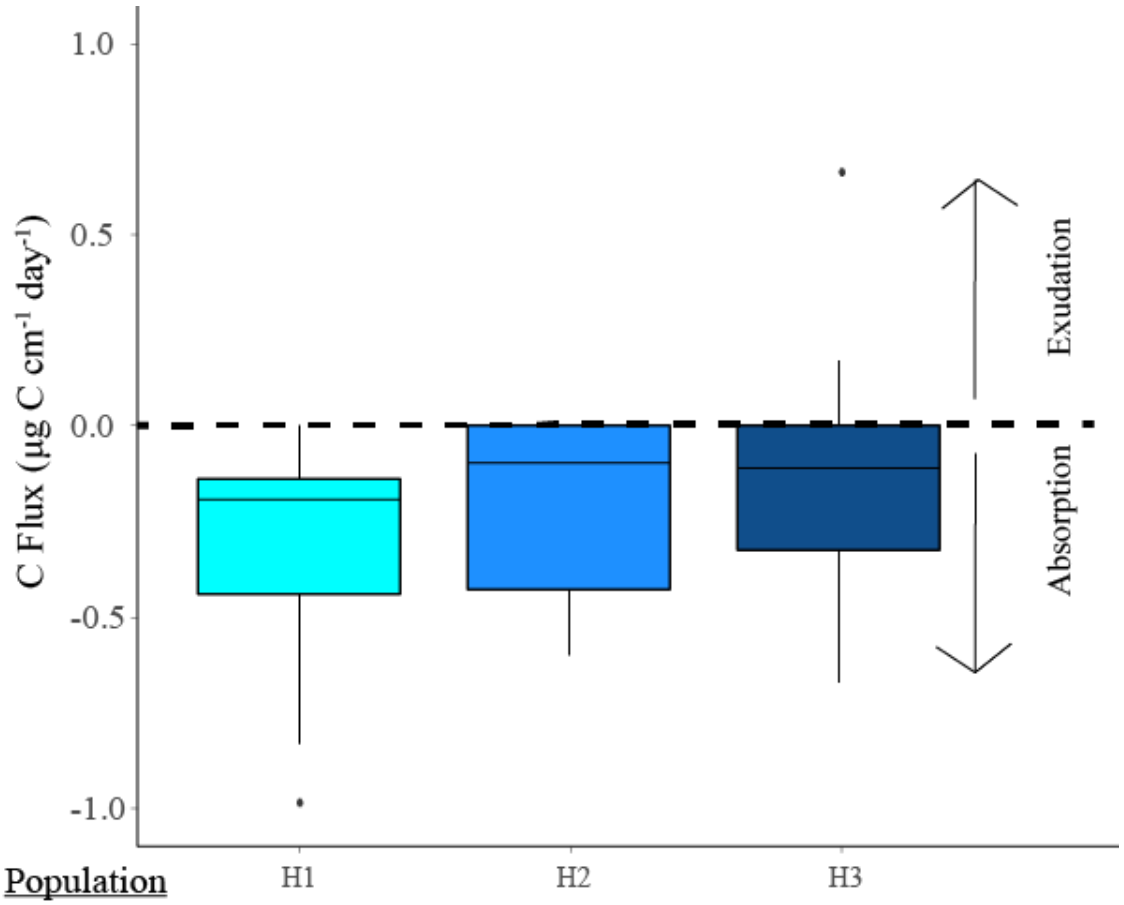

Figure 2. 6. Box and whisker plot indicating median, 25th and 75th percentiles as boxes and the 10th and 90th percentiles as error bars of fine root C flux (µg C cm^{-1} day^{-1}) of *Populus fremontii* measured at the High Elevation Garden.

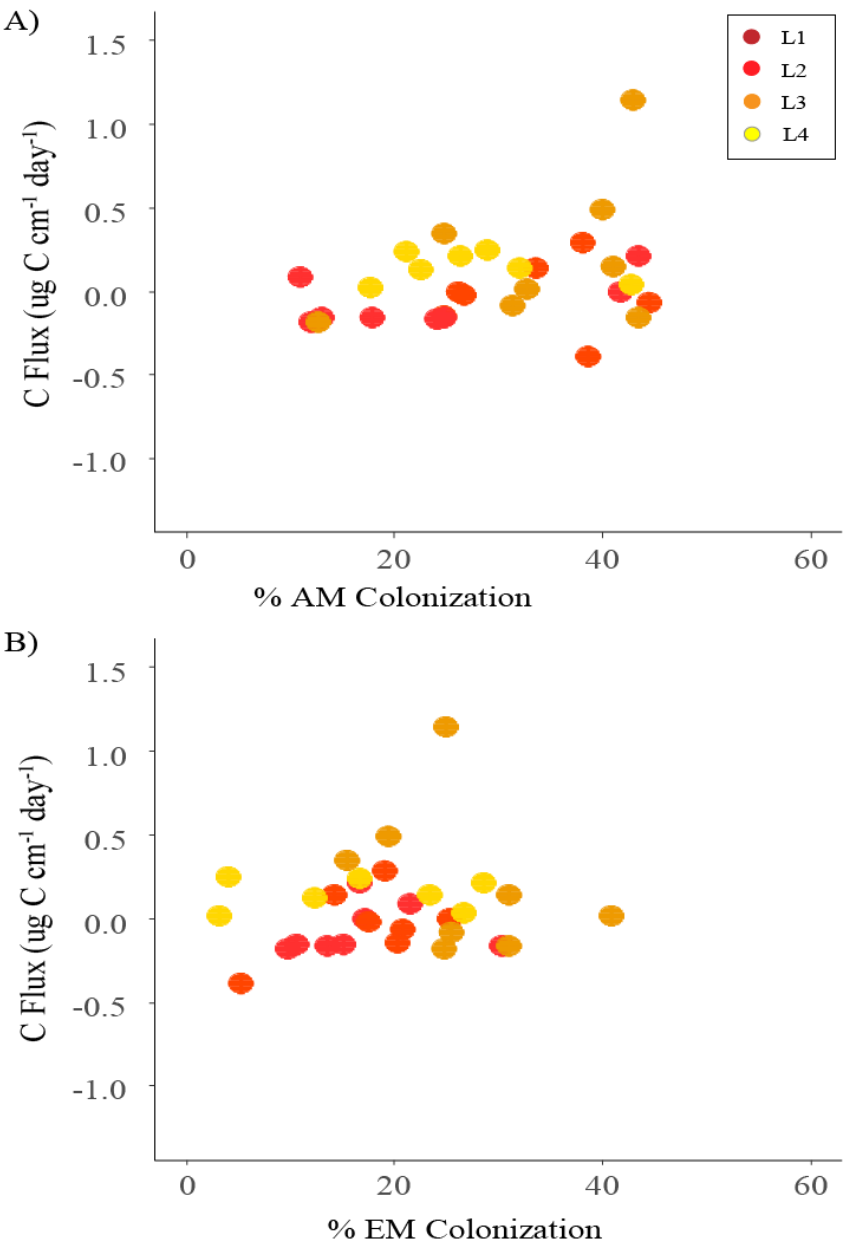

Figure 2. 7. Arbuscular (AM; panel A) and ectomycorrhizal (EM; panel B) colonization by fine root C flux rates (µg C cm^{-1} day^{-1}) of *Populus fremontii* at the Low Elevation Garden. No correlations were found between mycorrhizal colonization and C flux rates. Provenances coded by color.

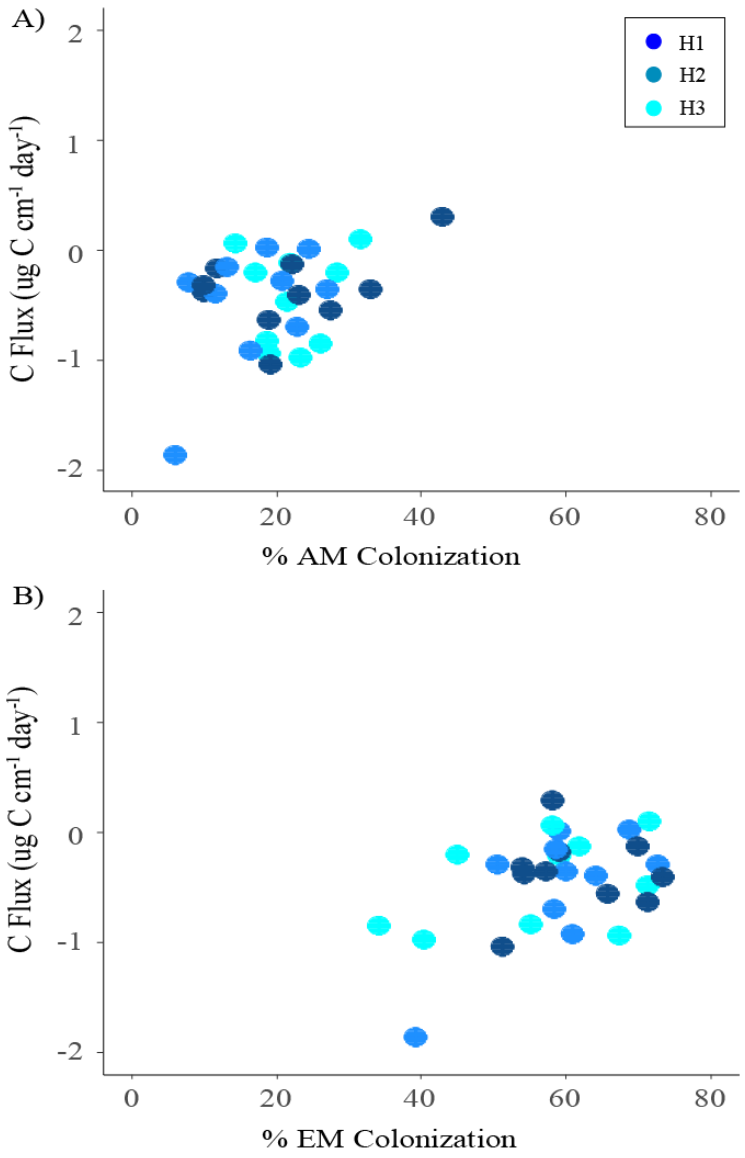

Figure 2. 8. Arbuscular (AM; panel A) and ectomycorrhizal (EM; panel B) colonization by C flux rates (µg C cm^{-1} day^{-1}) of *Populus fremontii* at the High Elevation Garden. No correlations were found between mycorrhizal colonization and C flux rates. Provenances coded by color.

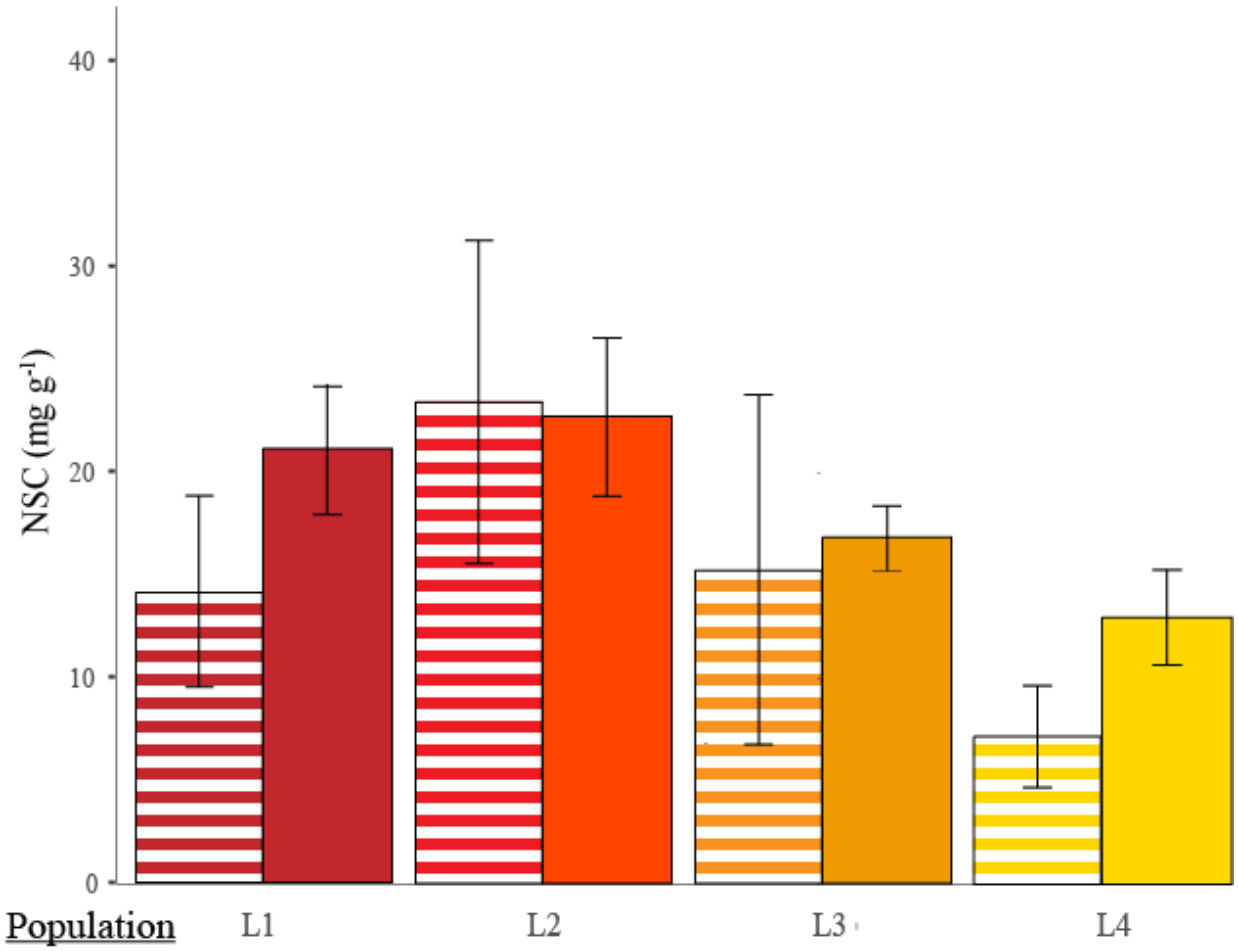

Figure 2. 9. Mean (± 1SE) non-structural carbohydrates (NSC (sugar (solid) and starch (lined))) (mg g^{-1} dry weight) of *Populus fremontii* roots at the Low Elevation Garden. No significant differences were found between NSC types within and across provenances.

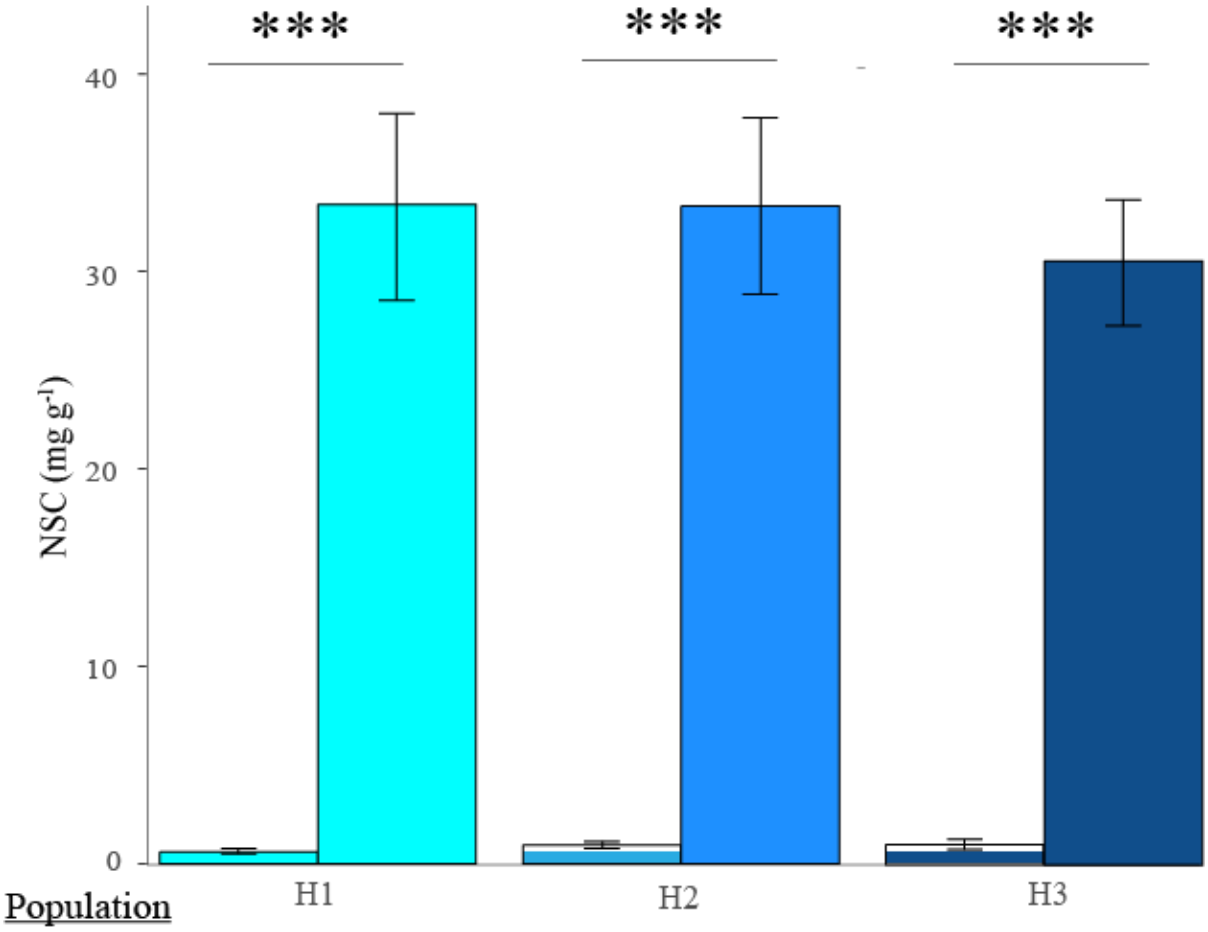

Figure 2. 10. Mean (± 1SE) non-structural carbohydrates (sugar (solid) and starch (lined)) (mg g^{-1} dry weight) of *Populus fremontii* measured at the High Elevation Garden. Lines indicate differences of NSC type within provenances (p-values are as follows: *** < 0.001).

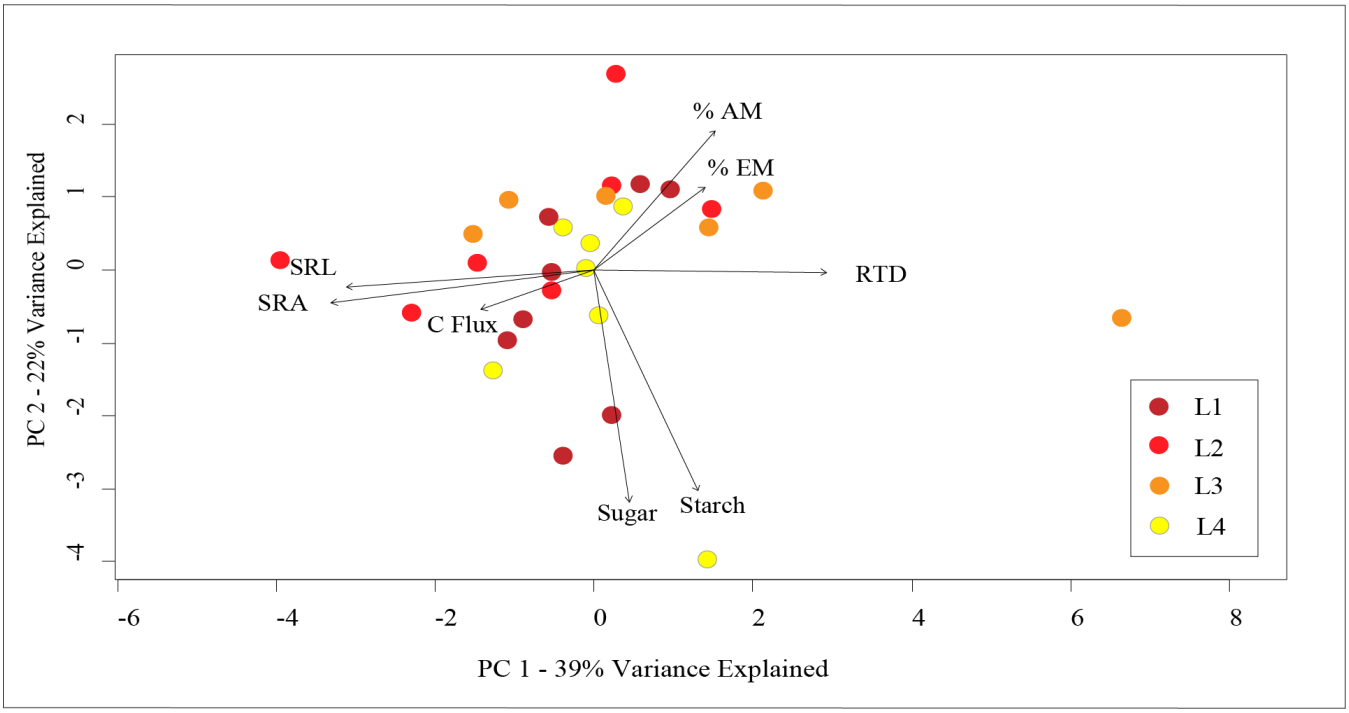

Figure 2. 11. Principal component analysis 1 (PCA 1) of root traits of *Populus fremontii* at the Low Elevation Garden. Specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 g^{-1}), fine root C flux (µg C cm^{-1} day^{-1}), percent mycorrhizal colonization (Arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD; g cm^{-3}), starch and sugar NSC concentrations (mg g^{-1}). Provenances coded by color. PC axis 1 explained 39% of the variance; PC axis 2 explained 22% of the variance (Table S2.9).

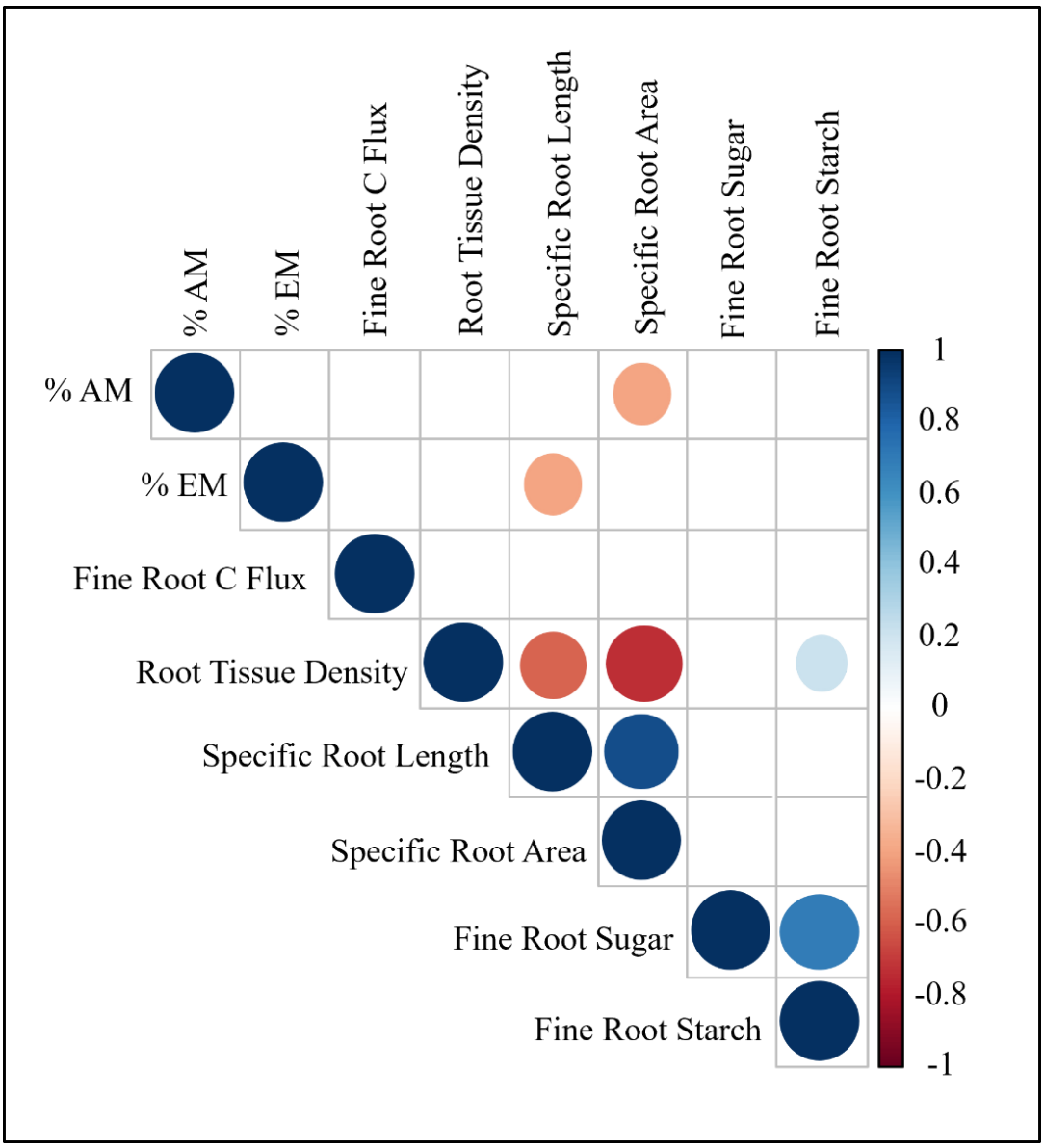

Figure 2. 12. Pearson's correlation matrix of *Populus fremontii* root traits at the Low Elevation Garden. Specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 g^{-1}), fine root C flux (µg C cm^{-1} day^{-1}), percent mycorrhizal colonization (Arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD; g cm^{-3}), starch and sugar NSC concentrations (mg g^{-1}). Only significant correlations appear (p-value < 0.05). Color indicates positive (blue) and negative (red) correlations with numbers correlated by side bar ranging from 1 to -1.

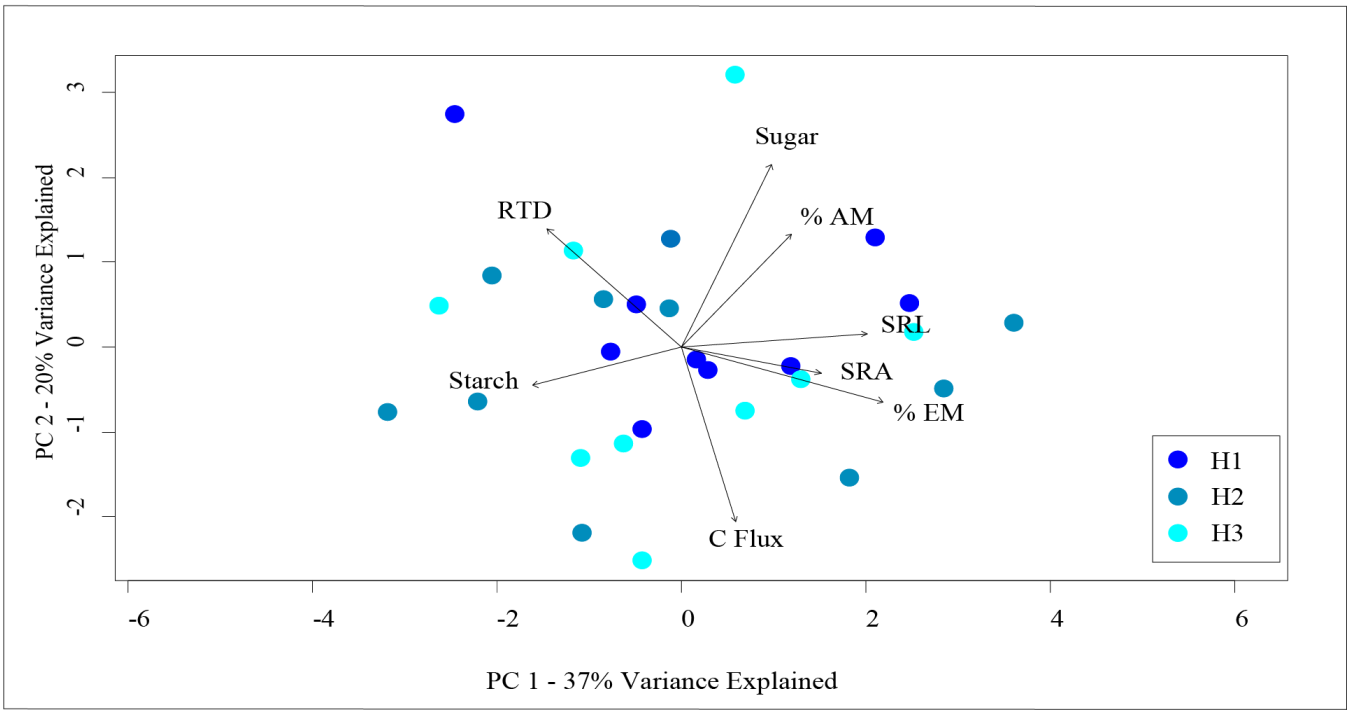

Figure 2. 13. Principal component analysis 2 (PCA 2) of root traits of *Populus fremontii* at the High Elevation Garden. Specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 g^{-1}), fine root C flux (µg C cm^{-1} day^{-1}), percent mycorrhizal colonization (Arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD; g cm^{-3}), starch and sugar NSC concentrations (mg g^{-1}). Provenances are coded by color. PC axis 1 explained 37% of the variance; PC axis 2 explained 20% of the variance (Table S2.10).

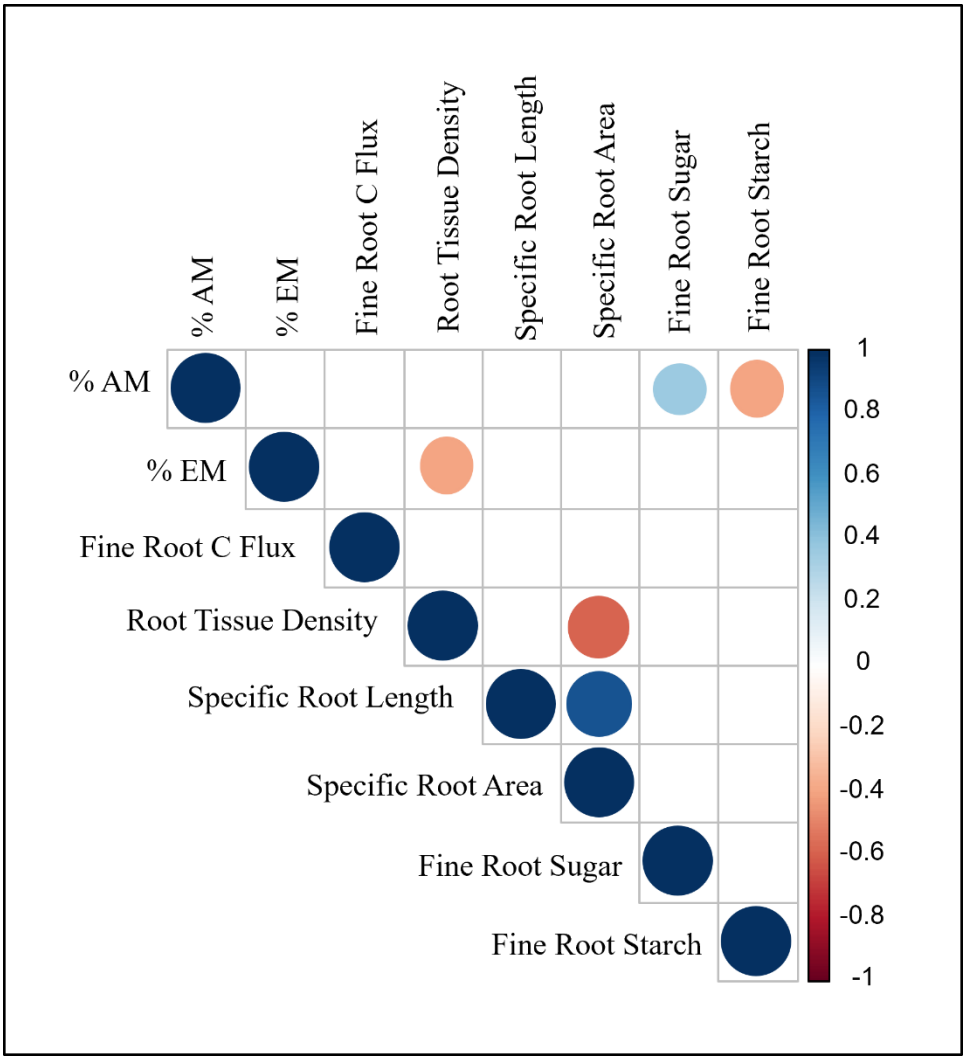

Figure 2. 14. Pearson's correlation matrix of *Populus fremontii* root traits at the High Elevation Garden. Specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 g^{-1}), fine root C flux (µg C cm^{-1} day^{-1}), percent mycorrhizal colonization (Arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD; g cm^{-3}), starch and sugar NSC concentrations (mg g^{-1}). Only significant correlations appear (p-value < 0.05). Color indicates positive (blue) and negative (red) correlations with numbers correlated by side bar ranging from 1 to -1.

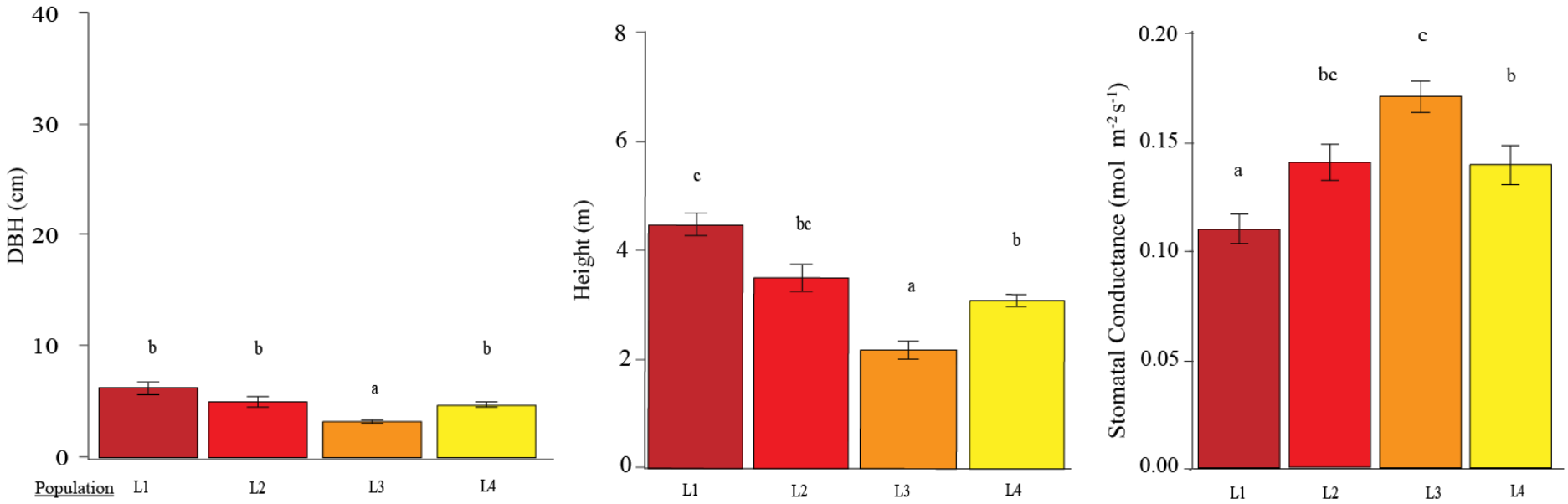

Figure 2. 15. Mean (± 1SE) aboveground traits of *Populus fremontii* at the Low Elevation Garden; diameter at breast height (DBH; cm), height (m) stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}). Letters indicate significant differences among provenances ($p < 0.05$).

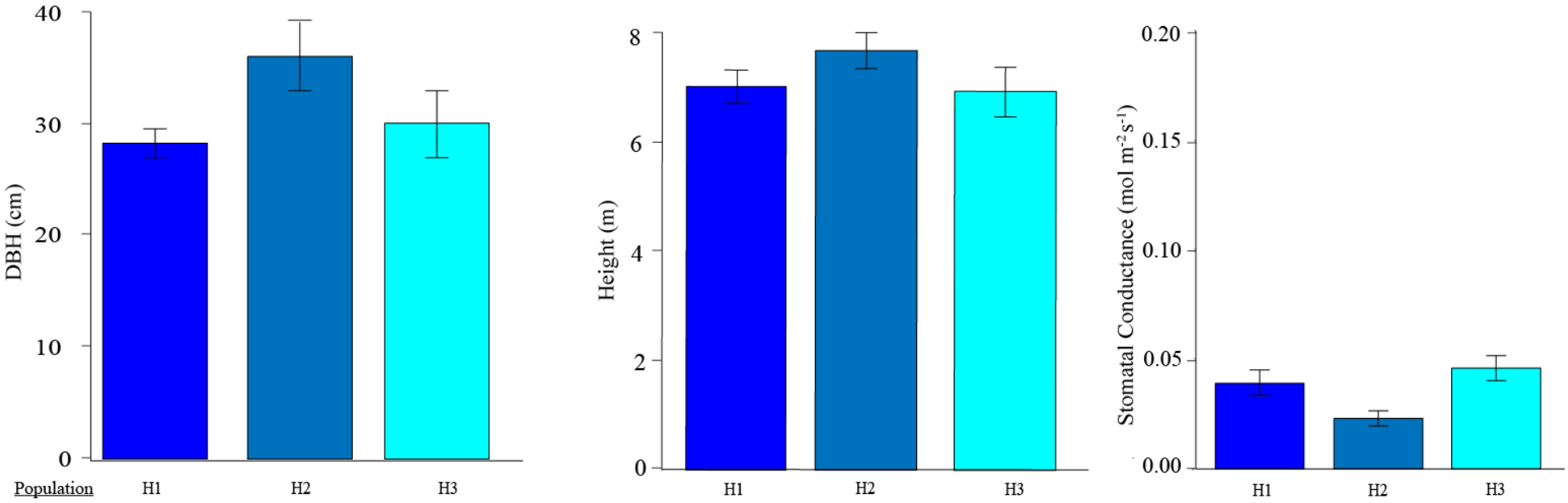

Figure 2. 16. Mean (± 1SE) aboveground traits of *Populus fremontii* at the High Elevation Garden; diameter at breast height (DBH; cm), height (m) stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}). No significant differences were found between aboveground traits within and across provenances.

Tables

Table 2. 1. Selected gardens and *Populus fremontii* provenances in the Southwest Experimental Garden Array (SEGA) included in the current study. Elevation and coordinates describe the origin for each provenance. Cuttings were taken in 2014 and transplanted into the SEGA. Mean annual temperature (MAT; °C). Provence and gardens located within ecotype range are as follows; bold – Sonoran Desert & italicized – Utah Plateau.

Origin Location	**Provenance**	**Elevation (m)**	**Latitude**	**Longitude**	**Garden**	**Number of trees sampled**
Bill Williams, Colorado, AZ	L1	143	34.27606	114.0585	LEG	7
Hassayampa, Wickenberg, AZ	L2	575	33.9088	112.6764	LEG	8
San Pedro, Charleston, AZ	L3	1219	31.61047	110.1669	LEG	7
Sonoita Creek Patagonia, AZ	L4	1234	31.5364	110.7631	LEG	8
Citadel Wash, Little Colorado Rv., AZ	H1	1299	35.613	111.319	HEG	10
Jack Rabbit, Little Colorado Rv., AZ	H2	1507	34.96	110.436	HEG	10
Keams Canyon, UT	H3	1920	35.81152	110.1696	HEG	10
Garden Location					**MAT**	
Yuma, Arizona	LEG	49	32.8498	114.4928	23.6	30
Canyonlands Research Center, UT	HEG	1581	38.0925	109.5978	11.6	30

Chapter 3: Linking above and belowground traits of a dual-colonized *Populus* species with adaptation to arid climates

3.1 Introduction

Arid environments, such as the U.S. southwest, are experiencing the effects of climate change with ever-escalating drought events (Seager et al., 2007), making water a limiting factor for tree growth within this region (Adams & Kolb, 2005). Decreased water supply and increased atmospheric vapor pressure deficit are typically associated with drought conditions. Consequently, in these situations, when the stomata open to assimilate carbon (C), it poses a higher risk of hydraulic failure for the tree, and therefore photosynthesis comes at an increased cost (Wilson et al., 2000). Trees that reside in arid environments could face tradeoffs in C investment above- or belowground (AG-BG) to tolerate or avoid stressful conditions induced by drought. For example, *Populus tremuloides* (Quaking Aspen) tolerate water stress by increasing C investment aboveground (AG) in the form of denser woody and leaf structures, thereby protecting their hydraulic systems from cavitation (Anderegg & Hillerislambers, 2016). In comparison, *Pinus ponderosa* (Ponderosa Pine) avoids water loss through stomatal closure decreasing photo assimilation of C (Anderegg & Hillerislambers, 2016). Belowground (BG), root trait adaptations to hot and dry conditions include increased exudation rates, stimulating microbial communities (Preece et al., 2018) and interaction with mycorrhizal fungi (Gehring et al., 2017). However, studies often view the consequences of AG-BG traits on ecosystem processes in isolation (Wardle, 2013). Separating stress adaptation of a tree species into AG-BG components could potentially ignore important feedback between each component or overestimate the role of one component's response in total fitness at the plant level. Furthermore, studies that have linked AG-BG responses find contrasting evidence of coordinated (G. Liu et

al., 2010) and partially decoupled (Freschet et al., 2013) AG-BG trait response to abiotic conditions indicating highly complex relationships between limiting environmental factors which may impact overall plant fitness. Studies evaluating intraspecific AG-BG traits simultaneously create opportunities to understand plant-level adaptation to marginal arid environments.

The foundation species, *Populus fremontii* (Fremont Cottonwood), is native to the arid U.S. Southwest, where it supports riparian habitats and its presence drives ecosystem processes such as litter decomposition and soil net N mineralization (Schweitzer et al., 2004). Unique ecotypes comprised of clustered provenances form this species' widespread geographical range (Ikeda et al., 2017). Individuals that make up these ecotypes are highly genetically consistent, with little genetic information shared between ecotypes (Ikeda et al., 2017). Common garden studies also indicate phenotypic plasticity within ecotypes in leaf phenology (budset), and between ecotypes in leaf economic traits (specific leaf area (SLA), stomatal density, etc.), whole-plant architecture (tree height, canopy area, etc.), and wood economic traits (mid-day water potential, bark percentage, etc.) (Blasini et al., 2021; Cooper et al., 2019; Hultine et al., 2020). Hence, these results from common gardens highlight diverse intraspecific trait variation AG among ecotypes across climate gradients. Although there is much research done on AG traits, knowledge of BG traits in this species remains limited. As a result, BG C allocation and trait variation among ecotypes are unknown, despite the broad implications these traits could have on ecosystem processes such as C cycling.

Aboveground, the movement of C from the atmosphere to plants begins in the leaves, which use light energy to synthesize reduced C compounds and further transfer these sugars to leaves, branches, and stems to maintain metabolic activities, growth, and reproduction. Belowground, the flow of C from aboveground organs into roots, and ultimately soils, can be

allocated in a variety of ways, including 1) building thick or fine roots to maintain structural integrity and explore the soil matrix to absorb water and nutrients, 2) releasing labile C into the rhizosphere, otherwise known as exudation, which affects nutrient cycling though priming of microorganisms that increase soil nutrient availability to plants (Finzi et al., 2015), and 3) associating with mycorrhizal fungi which, in exchange for sizeable amounts of plant-fixed C, provide their hosts with increased assess to water and nutrients within the soil environment, past the zone of depletion (Smith & Read, 2008). Association with mycorrhizal fungi can influence the root economic spectrum. Previous theories proposed the root economic spectrum as a counterpart to aboveground economic trait theories, such as the leaf economic spectrum, which describes fast and slow-growing species along a continuum of acquisitive to conservative traits, respectively (Reich, 2014). However, roots are unique in their association with symbiotic fungi, altering trait axes from a two-dimensional to a multi-dimensional space with two gradients: 1) the conservation gradient, which describes lifespan categorizing fast and slow growing species integrating C flux measurements, and 2) the collaboration gradient, which explains the trade-off of association with mycorrhizal fungi and building more efficient root systems (Bergmann et al., 2020; Wen et al., 2022). As a result, roots may "outsource" resource acquisition to their fungal partners instead of investing C into creating more efficient roots (Bergmann et al., 2020).

Most tree species form a single 'mycorrhizal type', namely ecto- or arbuscular mycorrhizas, and consequently, host species identity becomes a proxy for mycorrhizal type. However, the relationship between mycorrhizae and tree species is more nuanced than this, and some species can host two mycorrhizal types, otherwise known as dual-colonizing species (Teste et al., 2020). For example, *Populus fremontii* can associate with arbuscular and ectomycorrhizas simultaneously, making it a 'dual-colonized' species (Hultine et al., 2020; Meinhardt & Gehring,

2012; Chapter 2). As plants invest considerable portions of C to mycorrhizae and the interaction between each type and host roots is unique (Tedersoo & Bahram, 2019), dual-colonized species will be essential to disentangle impacts of mycorrhizal type on root economic trait space without the confounding factors of host identity and environment.

Ecto- (EM) and arbuscular mycorrhizal (AM) trees can have distinct effects on ecosystem processes such as nitrogen cycling and phosphorus mobilization (Phillips et al., 2013; Read & Perez-Moreno, 2003), litter decomposition (Brzostek et al., 2015), and soil C storage (Soudzilovskaia et al., 2019). Recent work demonstrates that *P. fremontii's* ecotypes differ in the dominant mycorrhizal type as measured by levels of AM and EM colonization (Hultine et al., 2020). These differences could mean that trees dominated by one mycorrhizal type could influence BG ecosystem processes differently than trees dominated by the other mycorrhizal type. Furthermore, the extent to which mycorrhizal type governs other root traits such as root morphology and exudation rates in a dual-colonizing species is unclear. Physical interaction with the roots varies greatly between the two types. Arbuscular mycorrhiza fungi live only within the root cortex, while EM also occupy the root cortex and/or epidermis, but also fungal tissue also covers fine root tips (Smith & Read, 2008), potentially creating thicker or thinner roots depending on colonization. Additionally, the C cost to the plant is higher for EM than AM associating trees, indicating larger portions of C sent to roots colonized in EM fungi (Tedersoo & Bahram, 2019). The characteristics of each mycorrhizal type could play a key role in their impact on root economic traits and downstream ecosystem processes. Moreover, dual-colonizing species could have considerable intraspecific variation in their root systems due to the dominant mycorrhizal type across their geographical range.

As a novel approach to understanding the influence of mycorrhizal type on BG root traits, which may have implications for whole-tree C allocation strategies, I use the dual-colonized tree species *P. fremontii* as a model system. In this study, I attempted to manipulate the dominance of EM and AM fungi colonizing roots while simultaneously reducing variability in abiotic conditions to gain a tree-level understanding of trait differentiation and C investment of *P. fremontii*. To do this, I estimated C uptake and allocation with a specific focus on mycorrhizal association in commonly measured AG-BG traits. I collected *P. fremontii* cuttings across provenances and planted them into pots in a greenhouse experiment to target three objectives: 1) understand if ecotype drives dominant mycorrhizal type, 2) understand if mycorrhizal type in a dual-colonized species will influence root traits across ecotypes, and 3) understand if there is a link between above- and belowground traits across ecotypes.

3.2 Methods

3.2.1 Collection of plant material

Between December 2020 and February 2021, I collected *Populus fremontii* (S. Waston) cuttings across intact natural riparian systems along an elevational gradient of approximately 1,500 to 2,400 m from southern Utah to western Arizona, USA. Each cutting (a stem cut at the base of small branch with a terminal bud) was taken from known mother trees before leaf flush to ensure healthy buds. Cuttings were transported to Northern Arizona University (NAU), where they were further trimmed, coated with Rhizopon AA® (New York, USA) to encourage root growth at the base of the stem, and placed into small 0.16 L D-16 pots (Stuewe & Sons, Tangent, USA) with Cornell peat mix, 1:1:1 ratio of sphagnum peat moss: horticultural perlite: coarse vermiculite potting mix from NAU Greenhouse facilities.

3.2.2 Mycorrhizal type manipulation

To culture the EM and AM fungal inoculum used to manipulate the mycorrhizal type of cuttings, I collected field soil from a natural population of *P. fremontii* located at Wet Beaver Creek, AZ USA, (34°39'48.6"N 111°43'09.4"W) in December 2020. This location contains fungal species from both mycorrhizal types (Meinhardt & Gehring, 2012). Field soils were collected at approximately 15–20 cm depth, sieved to remove roots and other organic matter, and cut with Turface™ for a final composition of 2/3 sieved field soil and 1/3 Turface™. The soil was subsequently used to inoculate six 5-gallon tubs, three of which were planted with *Allium porrum* and *Tagetes patula* to propagate only AM fungi, and the other three sowed with *Pinus ponderosa* seeds to propagate only EM fungi. These tubs were regularly watered to encourage colonization. Before transferring the cultured soils to pots containing the *P. fremontii* cuttings, roots of *Pinus ponderosa* were visually assessed to determine if they were colonized by EM

fungi. This method of 'soil-training' has been used before in the Gehring lab to directly manipulate plants' EM and AM colonization (Meinhardt & Gehring, 2012).

In July 2021, after roots developed, cuttings were transferred to larger 0.65 L D-40 pots (Stuewe & Sons, Tangent, USA), and each D40 pot was filled with 1/2 "play sand" and 1/2 field soil. To mitigate contamination from other soil microbes, the soil mixture was steam sterilized at 95 °C for 48 hours after combining sand and field soil. To each pot one of the following inoculation treatments were applied: (1) 'Control' – no inoculum added, (2) 'EM' – 0.25 g chopped *Pinus ponderosa* ectomycorrhizal roots (after two rinses with water), (3) 'AM'– 0.25 g chopped arbuscular mycorrhizal fine roots of *Allium porrum* and *Tagetes patula*, and (4) 'Dual' – equal parts of treatments for a total of 0.5 g (2) and (3). Inoculated cuttings grew for 12 weeks in the greenhouse. Racks of 6–8 cuttings of the same treatment were placed randomly on greenhouse benches and rotated periodically. Cuttings were placed under a 17-hour daylight timer and watered every other day till full saturation. The average temperature was 20°C and average relative humidity was approximately 40%.

3.2.3 Exudate collection and plant measurements

Approximately 12 weeks following transplanting and inoculation, I collected root exudates from the cuttings following the same methods presented in Chapter 2, with adjustments for the D-40 pots used in the greenhouse. Cuttings were gently shaken from pots, placed on a sterilized surface, and cleaned with 70% EtOH. Using forceps, a 15–20 cm section of root was separated from the root mass and covered with a moist KimWipe (Kimberly-Clark Professional, Roswell, USA). The cutting, root mass, and original soil mixture was reinserted into the D-40 pot with the selected covered root segment sticking out from the top. The exudation collection process hereafter mimicked that used under field conditions aside from the syringe system taped

to the rack holding each D-40 pot. Collection cuvettes were covered completely with aluminum foil for protection from light. I measured diameter at root crown (DRC; mm) at the first emerging root on the cutting stem. Height (cm) of each cutting was taken from DRC to the tallest apical meristem. Every living leaf was measured for total surface area and roots were cut from stems and saved in the freezer. Mass of stem, new growth as measured by bud scars, and roots were collected after 48–72 hours drying at 50 °C. All other measurements, including mycorrhizal colonization and leaf surface area were performed using the same methods described in Chapter 2 unless stated above. Non-structural carbohydrates were not analyzed for these samples.

3.2.4 Statistical Analysis

All statistical analysis was conducted in R studio (RStudio Team, 2022) with the following packages: plyr (Wickham, 2011), tidyr (Wickham and Henry, 2019), vegan (Oksanen et al., 2022), ape (Paradis and Schliep, 2019), ecodist (Goslee and Urban, 2007), and corrplot (Wei and Simko, 2021). All data was inspected visually through residual plots, and normality was assumed thereafter with no transformations made. Outliers were inspected by influential observations by Cook's distance and removed if greater than 1.

Mycorrhizal root inoculation did not result in the desired treatments, as cuttings from all treatments had high EM and AM colonization (Fig. 3. 1). There were no significant differences among inoculation treatments for either mycorrhizal type (Fig. 3. 1, Table S3.1). Therefore, I did not include inoculation treatment in the analysis and instead, I grouped cuttings based on created 'ecotypes. These ecotypes were defined by the climate of the source location and assigned into one of four groups: 1) 'cold & dry', 2) 'cold & wet', 3) 'warm & dry', and 4) 'warm & wet' (Fig. 3.2 & Table 3.1). Groupings were chosen based on climate data extracted from "ClimateNA v6.40" from 1991–2020 (Wang et al., 2016). I grouped cuttings in this way to explore how

similar source locations climate characteristics affected this species overall trait plasticity. A cluster analysis and principal component analysis (PCA) were preformed to group the populations into two categories based on mean annual temperature (MAT) and mean annual precipitation (MAP), to create four ecotypes (Table 3.1.).

Analysis of variance (ANOVA) was run for each measured variable at the ecotype level. Additionally, an ANOVA was preformed to examine differences among ecotypes, time periods, and the daily average of stomatal conductance values. To understand ecotype characteristics, a Principal component analysis (PCA) was run on BG traits including: (SRL (m g^{-1}), SRA (m^2 g^{-1}), fine root C flux, mycorrhizal colonization, and RTD (g cm^{-3})). A second PCA was performed to understand the relationship between AG (specific leaf area (SLA (m^2 g^{-1})), total shoot biomass (g), new shoot biomass (g), g_s (mol H_2O m^{-2} s^{-1})) and belowground traits. A Pearson's correlation matrix was then produced to examine significant relationships from each PCA.

To examine variation in combined traits among ecotypes, three permutational multivariate analysis of variances (PERMANOVA) were performed for the following three groupings; 1) aboveground, 2) belowground, and 3) combined aboveground and belowground.

3.3 Results

Belowground traits

3.3.1 Dominant mycorrhizal type across ecotypes

There was no difference in AM or EM colonization across ecotypes (Figure 3. 3., Table S3.2). When aggregated, average EM colonization for all ecotypes was approximately five times greater than AM colonization (3.6 and 22 percent, respectively ($t(-7)=56$, $p < 0.0001$). Within each ecotype, EM colonization was significantly higher than AM colonization for all ecotypes(Figure 3.3) . The 'Warm & Dry' ecotype had marginally higher EM than AM colonization ($t(-2)=8$, $p < 0.08$).

3.3.2 Root morphological, chemical, and mycorrhizal relationships across ecotypes

When all root traits were combined in multivariate space ecotypes did not differ significantly (Table S3.11). When ecotypes were combined, four patterns emerged (Fig. 3. 4. & 3. 5., Table S3.4). The first and strongest pattern was a positive correlation between SRL and SRA. The second was a negative correlation between SRA and RTD. The third was a negative relationship between SRL and RTD. The last was a negative relationship between SRL with percent EM colonization. No significant patterns emerged between AM colonization or C flux with any other root trait (Fig. 3. 5., Table S3.4).

3.3.3 Root chemical and morphological traits

When viewed in isolation, RTD and total root biomass were significantly different between 'Cold' ecotypes (Table 3.2., Table S3.3). 'Cold & Dry' was 62 and 76 percent greater than 'Cold & Wet' ecotype for RTD and root biomass, respectively. The 'Warm' ecotypes did not significantly differ from each other in any trait. Consistent with field measurements of

Chapter 2, fine root C flux values for cuttings in the greenhouse ranged from negative to positive (-0.72 to 2.8 μg C cm^{-1} day^{-1}) but were low. Mean C flux was 0.026 μg C cm^{-1} day^{-1} (±0.11), representing a positive C flux, i.e., exudation. There was no significant difference in C flux among ecotypes (Fig. 3. 3., Table S3.5).

Above- and belowground trait relationships

When viewed in multivariate space, AG-BG traits were linked in three major patterns (Fig. 3. 7. & 3. 8., Table S3.6). The first pattern was that total shoot biomass, total root biomass, and root tissue density were all positively correlated. Next, specific leaf area, specific root length, and specific root area were all positively correlated. Moreover, the traits described in the first pattern were negatively related to those in the second pattern. Third, EM colonization was negatively related to C flux and specific root length. No significant correlations were found between stomatal conductance, new shoot biomass, and AM colonization with any other trait. Additionally, ecotypes did not differ significantly in multivariate space of AG-BG traits (Table S3.11).

Aboveground traits

Shoot biomass significantly differed among ecotypes (Table 3. 3., Table S3.7). 'Dry' ecotypes were on average 70% heavier than 'Wet' ecotypes (Table 3. 2., Table S3.7). Additionally, DRC differed among ecotypes (Table 3. 2., Table S3.7). 'Cold & Dry' cuttings were 27% larger than 'Warm & Wet'. There were no differences among ecotypes in new growth or height (Table 3. 2., Table S3.7). In addition, SLA was on average 58% larger for 'Wet' ecotypes (Table 3.2, Table S3.7). There were no differences among ecotypes in new growth or height (Table 3. 2., Table S3.7).

Average daily stomatal conductance ranged from 0.0101 to 0.27 ($\bar{x} = 0.081 \pm 0.0081$) (mol H_2O m^{-2} s^{-1}). There were no differences between daily averages of stomatal conductance rates among ecotypes (Table S3.8). However, within ecotypes, there was significant difference in stomatal conductance rates among time periods (Fig. 3. 6., Table S3.9). For each ecotype, stomatal conductance was, on average, 73% lower at 3 PM than 9 AM. Within each time period there was no difference among ecotypes (Fig. 3. 6., Table S3.10). When combined in multivariate space ecotypes did not significantly vary (Table S3.11).

3.4 Discussion

3.4.1 Ectomycorrhizas dominated across ecotypes

To understand how mycorrhizal type dominance coincided with ecotypes, I measured the percent colonization of both AM and EM fungi on roots of *P. fremontii* for four climate-grouped ecotypes. Evidence of both mycorrhizal types, through repeated microscopy measurements, verifies *P. fremontii*'s dual-mycorrhizal status. Consistent with previous field and greenhouse observations, my study further confirms this species' association with two mycorrhizal types across age groups and climatic conditions (Hultine et al., 2020; Chapter 2). Across the four ecotypes, EM fungi dominated root colonization, and AM colonization was relatively lower. While the ecotypes did not differ in either AM or EM fungal colonization, within both 'Cold' groups and the 'Warm & Wet' ecotype, EM was significantly higher than AM colonization. Due to the presence of hyphae and vesicles in sampled roots, we can assume that AM fungi were present in the soil despite low colonization.

Previous work with dual-colonized genera, such as *Eucalyptus,* has found AM dominance at early life stages in both native (Chen et al., 2000) and non-native habitats (de Mendonça Bellei et al., 1992). Interestingly, it was EM and not AM fungi that primarily colonized roots, which may indicate a preference of these ecotypes for EM fungi in well-watered, high light conditions, such as a greenhouse. The source elevations from all ecotypes are relatively high (1587–2439 m), and across different *in-situ* surveys, EM associations were dominant at a higher elevation (1581 m) compared to lower elevation (49 m) site (Chapter 2). However, without cuttings taken from lower elevation ecotypes, I could not attribute high rates of EM colonization to elevation *per se.* At the elevation range considered, ecotype origin did not seem to drive AM or EM mycorrhizal dominance in the greenhouse study.

3.4.2 Carbon flux and specific root length correlated with ectomycorrhizal colonization but not arbuscular mycorrhizal colonization

Using a model species, *P. fremontii,* this study investigated root morphological and physiological traits without the confounding effects of host species with mycorrhizal type. Two root traits, fine root C flux and SRL, negatively correlated with EM colonization. Consistent with field observations (Chapter 2), the negative association between SRL and EM colonization indicates that the presence of ectomycorrhizas coincides with fine root morphology. I found that fine roots with high EM colonization are thicker than those with less EM colonization. Root trait variation of resource-acquisition strategies that incorporate mycorrhizal colonization and fine root C flux position mycorrhizal colonization and SRL on the 'collaboration' end of the gradient, where high mycorrhizal colonization reflects an 'outsourcing' strategy of roots to acquire nutrients (Wen et al., 2022). High values of SRL are positioned on the other end of the gradient, indicating roots of this type have a 'do-it-yourself" strategy to obtain nutrients (Wen et al., 2022). My results match this collaboration gradient, supporting the "collaboration" theory between plant roots and mycorrhizal association. Additionally, this theory includes another separate "conservation" gradient, which is placed orthogonal to the "collaboration" gradient, mapping traits on a fast and slow root economic spectrum based on C flux rates, where plants with high rates of C exudation are typically in nutrient-poor soils and associated with fast growth strategies (Wen et al., 2022). However, these gradients were established on traits collected over a wide range of taxa, and more nuanced traits, such as C-flux, could be highly variable across genera, potentially influencing gradient positions.

While EM fungal association correlated with two root traits, AM association was not significantly associated with any measured trait. This could be because overall AM colonization

was low and therefore the little fungal interaction with roots was not enough to illicit any change to its morphology or physiology. Previous results with high colonization rates in this species indicate negative correlations between AM fungal abundance and one morphological root trait, SRA (Chapter 2). Moreover, studies of other tree taxa that quantify AM abundance in roots do not show consistent correlations between root morphological traits, such as SRL, and AM colonization (Eissenstat et al., 2015; B. Liu et al., 2015; Yaffar et al., 2021; Zangaro et al., 2008). This inconsistency between previous work on *P. fremontii* and across various other tree species could highlight the context dependency of these relationships. Future work using non-colonized or uninoculated roots could disentangle colonization rates from frequently measured root traits.

3.4.3 Root tissue density separates ecotypes

When root traits were combined in multivariate space, ecotypes were separated by variation in RTD, with 'Dry' ecotypes having higher values than 'Wet' ecotypes. Higher RTD is often associated with slow growth limited by resource availability (e.g., water), therefore facilitating conservative growth strategies (Reich, 2014; Wen et al., 2022). The average precipitation is twice as high for the 'Wet' ecotypes than the 'Dry', meaning the 'Wet' ecotypes might not be limited by drought, therefore able to photosynthesize with little consequence with respect to water loss. Increased tissue density both AG-BG could be a strategy to resist hydraulic failure as both AG-BG tissue is at risk for xylem cavitation and embolism (Hacke et al., 2001; Hultine et al., 2006). Despite identical watering regimes in the greenhouse, variation of RTD among ecotypes was still present, indicating this might be a heritable trait of 'Dry' ecotypes. Furthermore, the separation of ecotypes by the morphological trait, RTD, could explain a critical strategy for this species in dry conditions.

3.4.4 Coordinated aboveground and belowground traits across ecotypes

In this study, I show coordination between AG-BG traits and intraspecific variation across four high elevation ecotypes. Previous work has established highly variable traits between ecotypes; however, these were isolated to either AG or BG traits of *Populus fremontii* (Cooper et al., 2019; Hultine et al., 2020). By considering AG-BG traits simultaneously, I investigated tree-level patterns and their variability across this species' high elevation range. Two different growth strategies between ecotypes appeared when AG-BG traits were combined in multivariate space. The first strategy is high aboveground biomass, and root tissue density exhibited by 'Dry' ecotypes. With a more conservative growth strategy associated with slow growth, 'Dry' ecotypes were clustered together when combined in a multivariate space. The second strategy is fast and competitive growth, reflected by cuttings with higher SRL and SLA in the 'Wet' ecotypes. These distinct growth strategies are possibly the result of differences in MAP and its impact on resource limitation (i.e., C acquisition through photosynthesis). Ecotypes adapted to drier conditions (more resource limited) had similar growth strategies but were different than those from wetter sites (less resources limited). These results show that intraspecific variation between the two temperature ecotypes results from their competitive or conservative growth strategies which manifest in the form of C allocation. High SRL and SLA are features of tree organs that maximize function with minimal carbon investment but decrease the longevity of said organs (Reich, 2014). For example, high SLA is correlated with increased leaf nitrogen content and therefore photosynthetic capacity (Reich et al., 1998). In comparison, high RTD requires a higher C investment for increased structure at the expense of reduced resource return (Bergmann et al., 2020). In summary, intraspecific variation of *P. fremontii* in root and leaf functional traits was likely driven by source MAP, and "Wet" and "Dry" ecotypes exhibited competitive and

conservative traits, respectively. These results show coordination between AG-BG traits, as reflected by high root and leaf economic traits. Future studies should attempt to manipulate abiotic conditions such as light availability, temperature, and precipitation to take the next step in investigating adaptation of these ecotypes to climate gradients.

3.4.5 Conclusions

In this study, I used a dual-colonized tree species to understand mycorrhizal association and root morphological and physiological trait variation across four, high elevation ecotypes. My results do not indicate ecotype drives mycorrhizal colonization of either functional type, AM or EM. I investigated mycorrhizal type and its influence over commonly measured root traits. Consistent with results from a previous field experiment, EM colonization was negatively correlated with a root morphological trait, SRL. Additionally, this work demonstrated a negative correlation between EM abundance and root C flux values, an interaction that was not seen in field observations. The data did not link AM colonization with any measured root trait, which could be due to overall low colonization of this mycorrhizal type across samples. In addition, I linked AG-BG growth strategies to show separate coordinated traits associated with ecotypes originating from areas with similar precipitation. In conclusion, high intraspecific trait variation in this species indicates elevated plasticity throughout this species range, which could be important as climate conditions continue to change.

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Figures

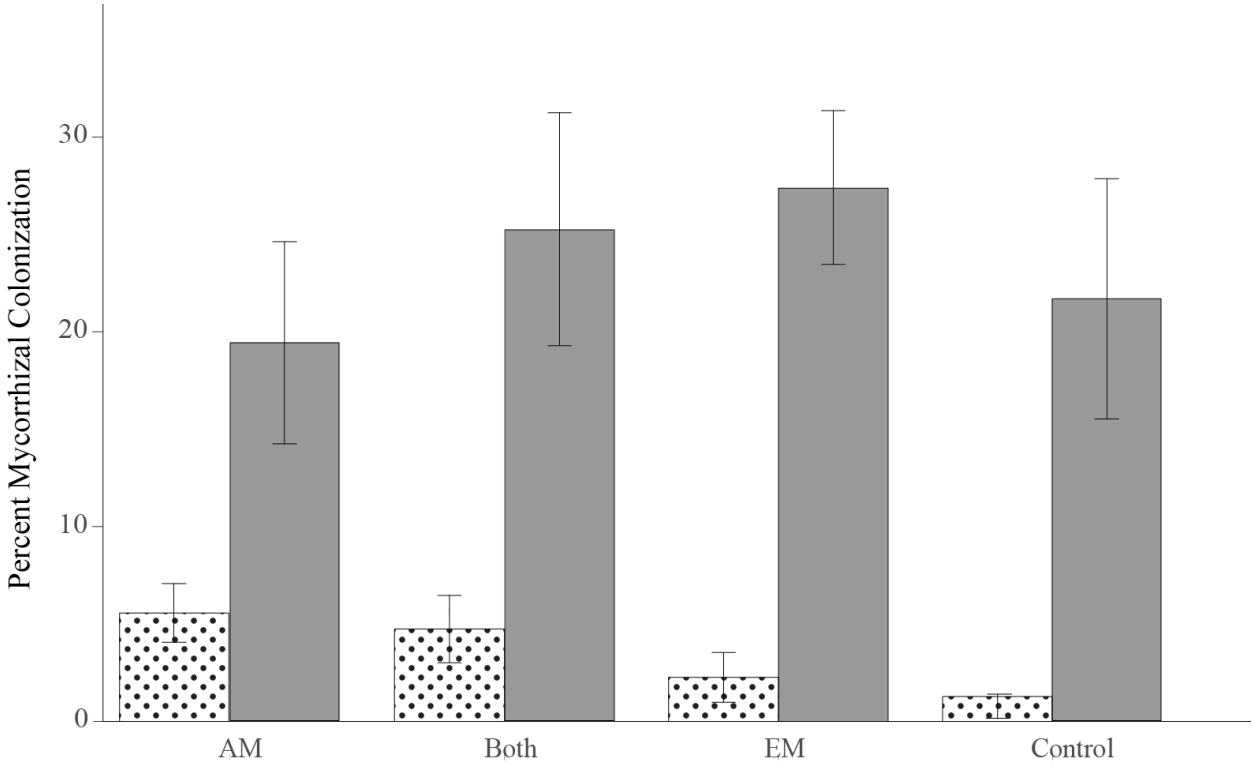

Figure 3. 1. Mean (± 1SE) ectomycorrhizal (EM: solid) and arbuscular mycorrhizal (AM: dotted) colonization of *Populus fremontii* roots across four inoculum treatments: 1) AM only, 2) Both – AM and EM, 3) EM only, and 4) Control – No mycorrhizal inoculum. There were no significant differences across treatments.

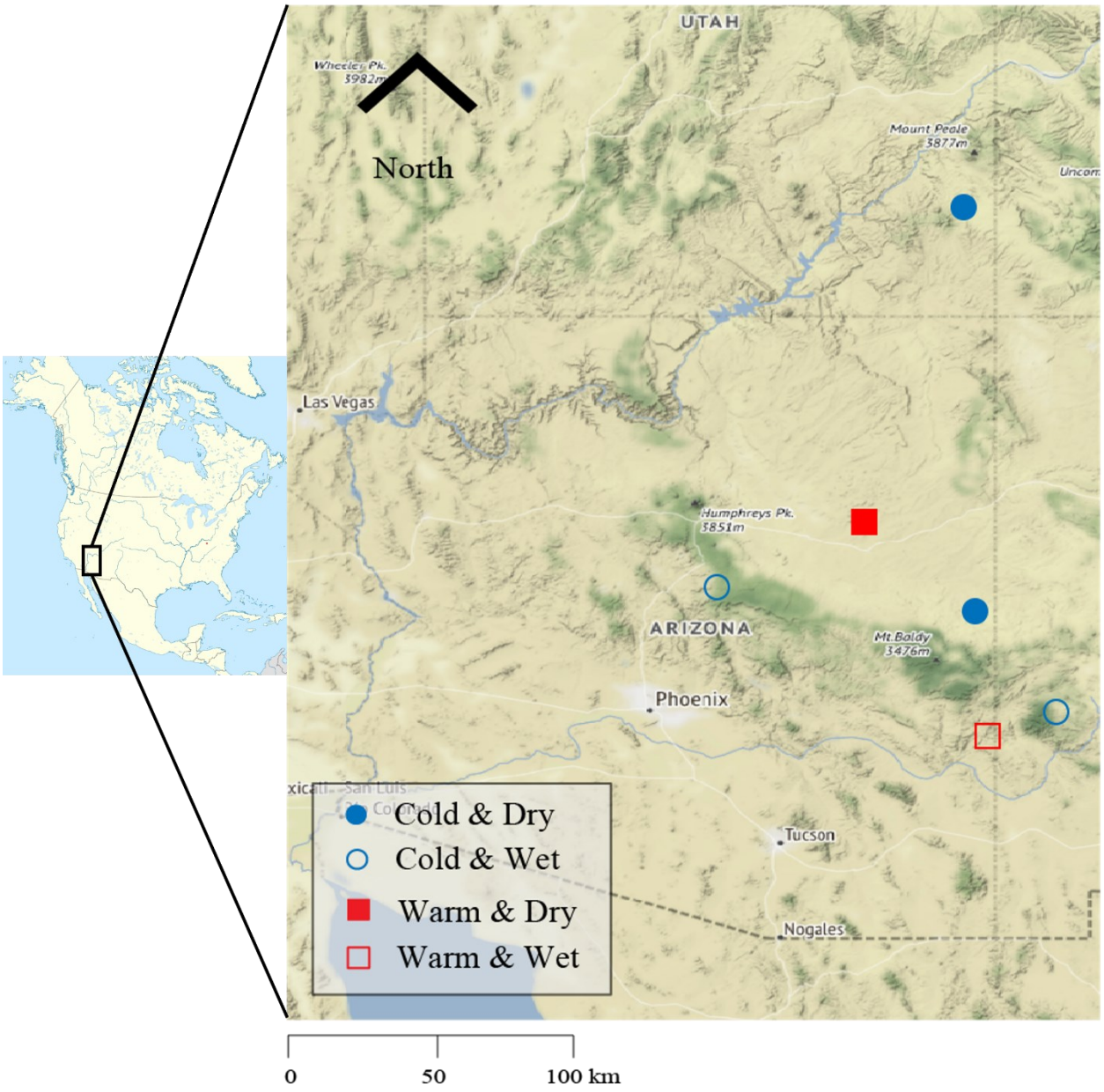

Figure 3. 2. Location of *Populus fremontii* cuttings collected from six source provenances coded by ecotype ('Cold' (blue) & 'Warm' (red); 'Dry' (solid) & 'Wet' (hollow)). See Table 3.1. for provenance information.

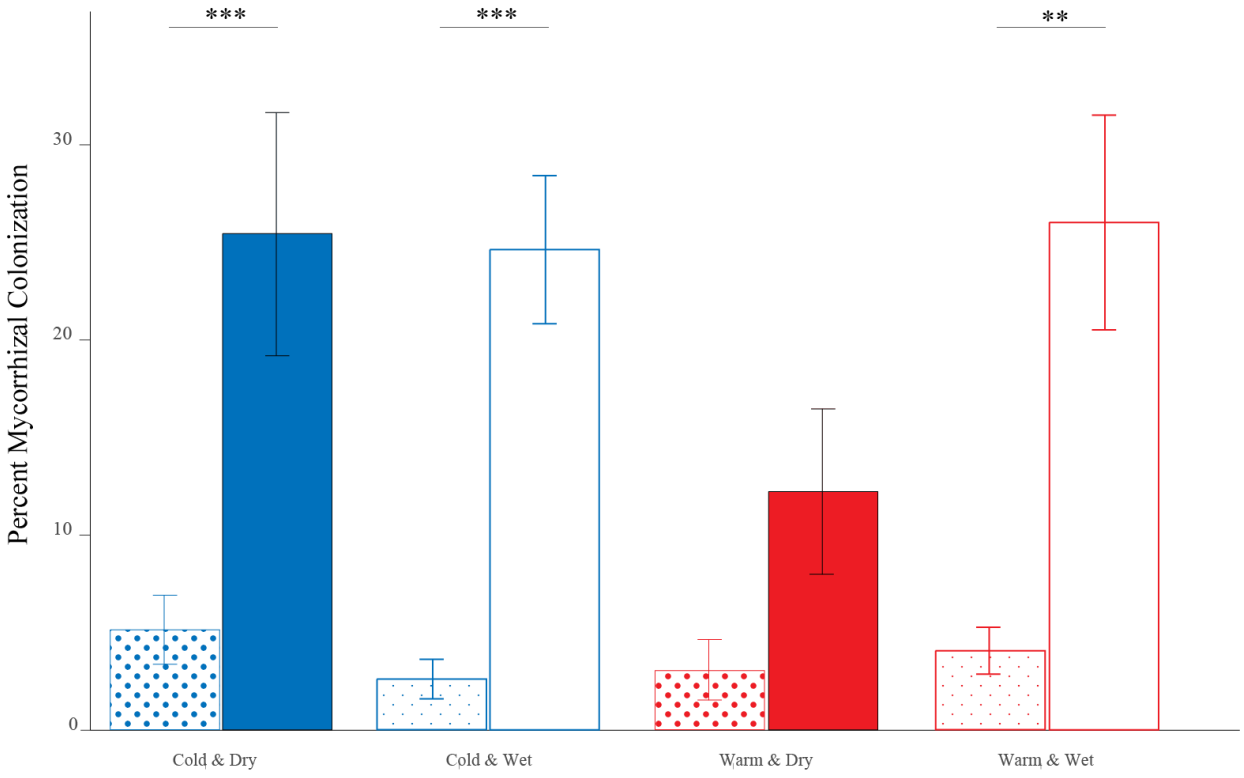

Figure 3. 3. Mean (± 1SE) mycorrhizal colonization of *Populus fremontii* by arbuscular (dotted) and ectomycorrhizal (solid) fungi from four ecotypes coded by color ('Cold' (blue) & 'Warm' (red); 'Dry' (solid) & 'Wet' (white)). Lines indicate differences within provenances (p-values are as follows: ** < 0.01, *** < 0.001).

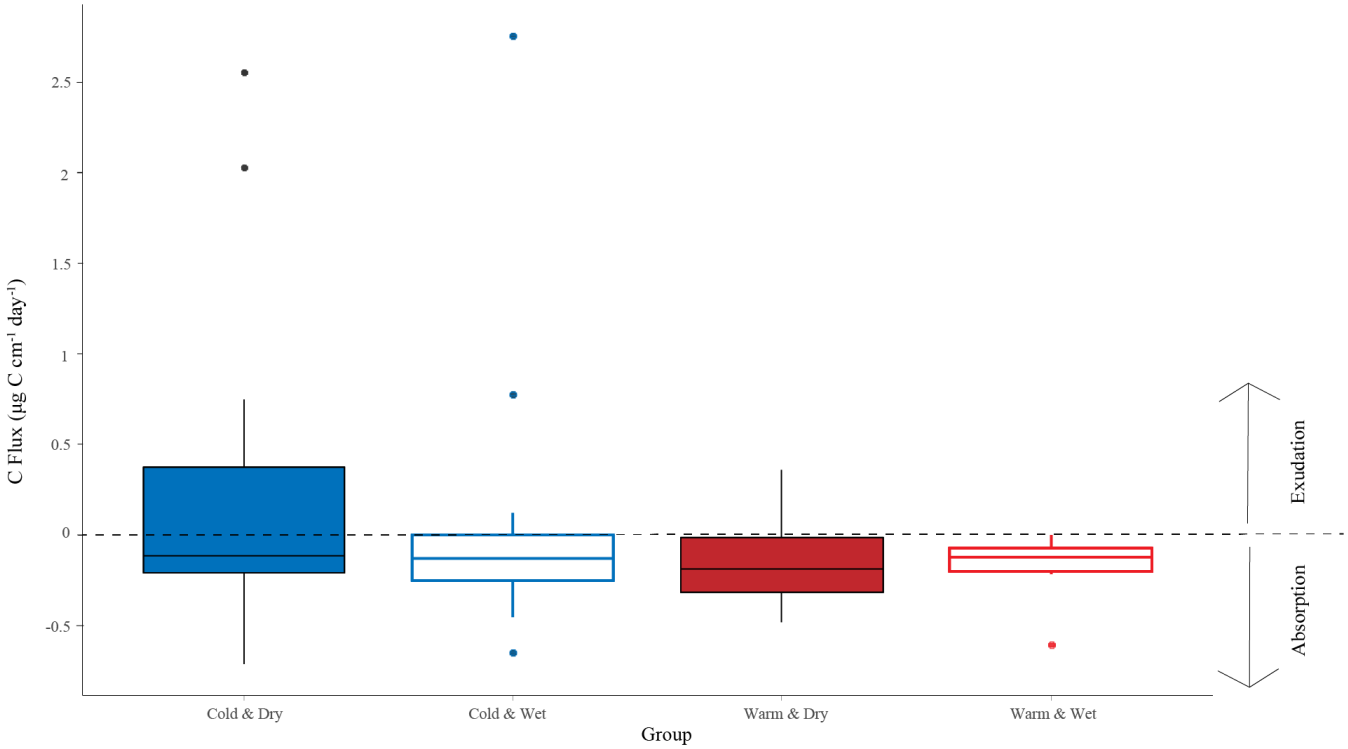

Figure 3. 4. Box and whisker plot indicating median, 25th and 75th percentiles as boxes and the 10th and 90th percentiles as error bars of fine root C flux (µg c cm^{-1} day^{-1}) of *Populus fremontii* across ecotype coded by color ('Cold' (blue) & 'Warm' (red); 'Dry' (solid) & 'Wet' (white)). There were no significant differences between ecotypes.

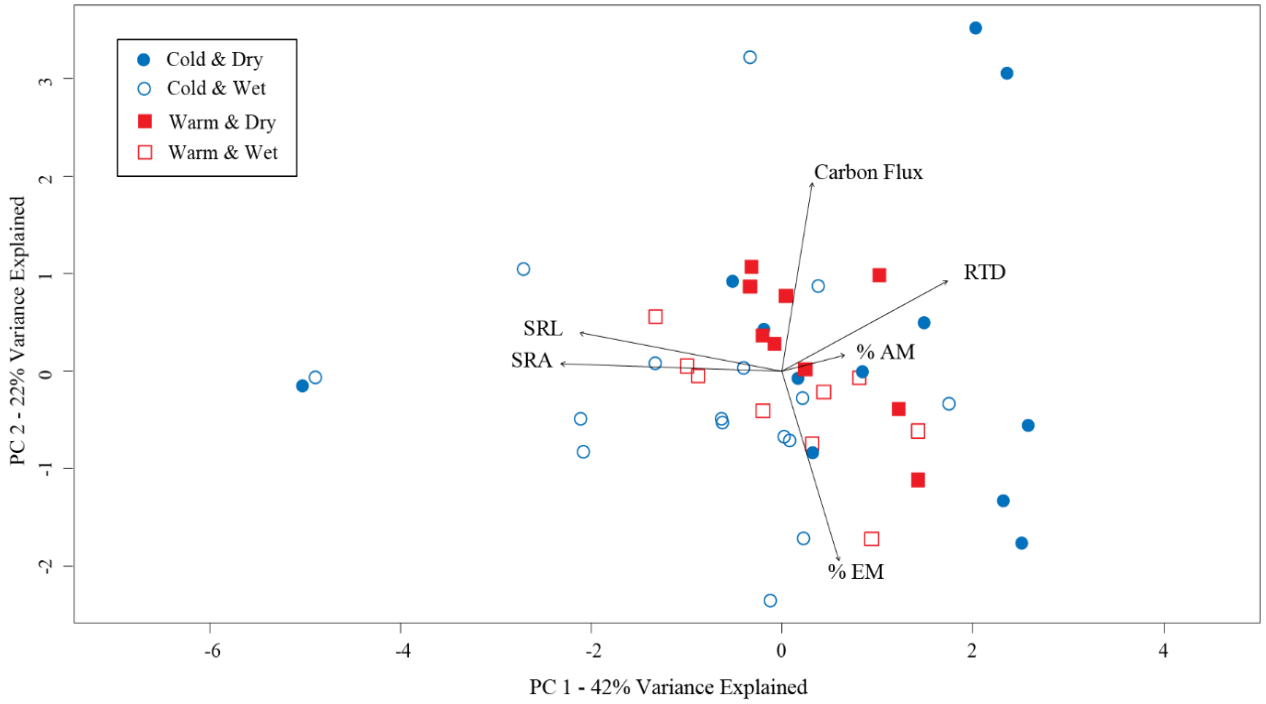

Figure 3. 5. Principal component analysis of root traits of *Populus fremontii*; specific root length (SRL (m g^{-1}), specific root area (SRA (m^2 g^{-1})), fine root C flux (µg C cm^{-1} day^{-1}), percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD (g cm^{-3})). Each point represents one cutting. Climate groupings are coded by color ('Cold' (blue) & 'Warm' (red); 'Dry' (solid) & 'Wet' (white)). PC axis 1 explained 42% of the variance; PC axis 2 explained 22% of the variance (see Table S3.4).

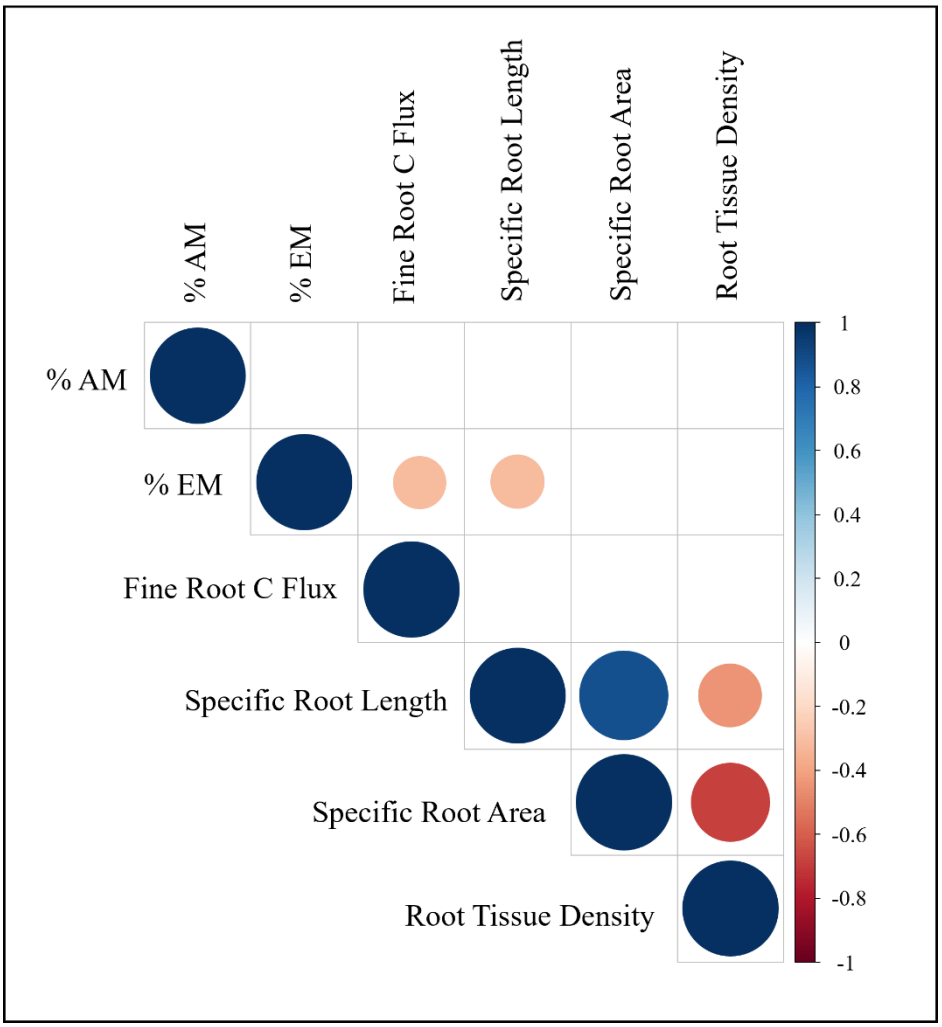

Figure 3. 6. Pearson's correlation matrix of *Populus fremontii* root traits; specific root length (SRL (m g^{-1})), specific root area (SRA (m^2 g^{-1})), fine root C flux (µg C cm^{-1} day^{-1}), percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD (g cm^{-3})). Only significant correlations appear (p - value < 0.05). Color indicates positive (blue) and negative (red) correlations with numbers correlated by side bar ranging from 1 to -1.

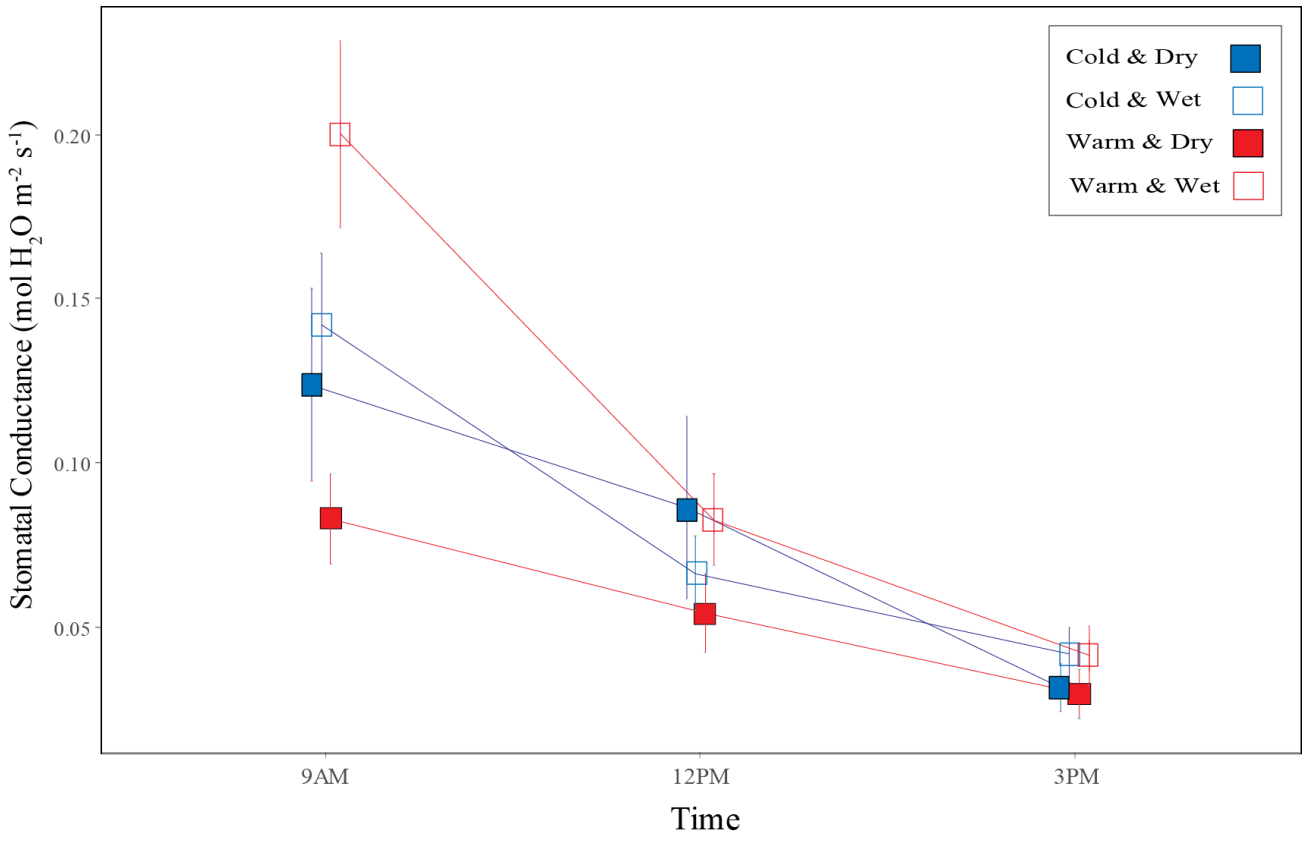

Figure 3. 7. Mean (± 1SE) stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}) across three time periods (9 AM, 12 PM & 3 PM) of *Populus fremontii* leaves. Ecotypes are coded by color ('Cold' (blue) & 'Warm' (red); 'Dry' (solid) & 'Wet' (white)).

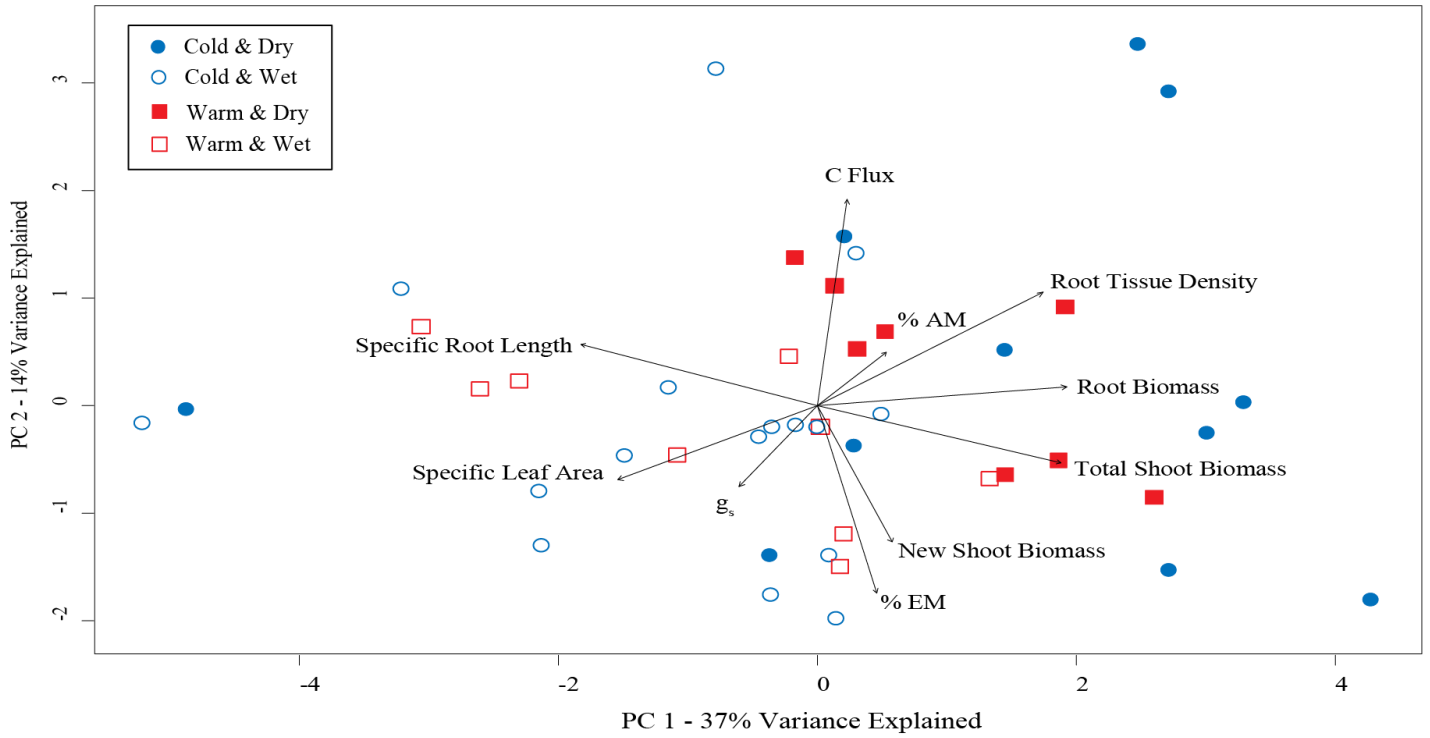

Figure 3. 8. Principal component analysis of aboveground (specific leaf area (m^2 g^{-1}), total shoot biomass (g), new shoot biomass (g), stomatal conductance (g_s (mol H_2O m^{-2} s^{-1})) and belowground traits (root biomass (g), specific root length (m g^{-1}), specific root area(m^2 g^{-1}), fine root C flux (µg C cm^{-1} day^{-1}), mycorrhizal colonization, root tissue density (g cm^{-3})) of *Populus fremontii*. Each point represents one cutting. Ecotypes are coded by color ('Cold' (blue) & 'Warm' (red); 'Dry' (solid) & 'Wet' (white)). PC axis 1 explained 37% of the variance; PC axis 2 explained 14% of the variance (see Table S3.6).

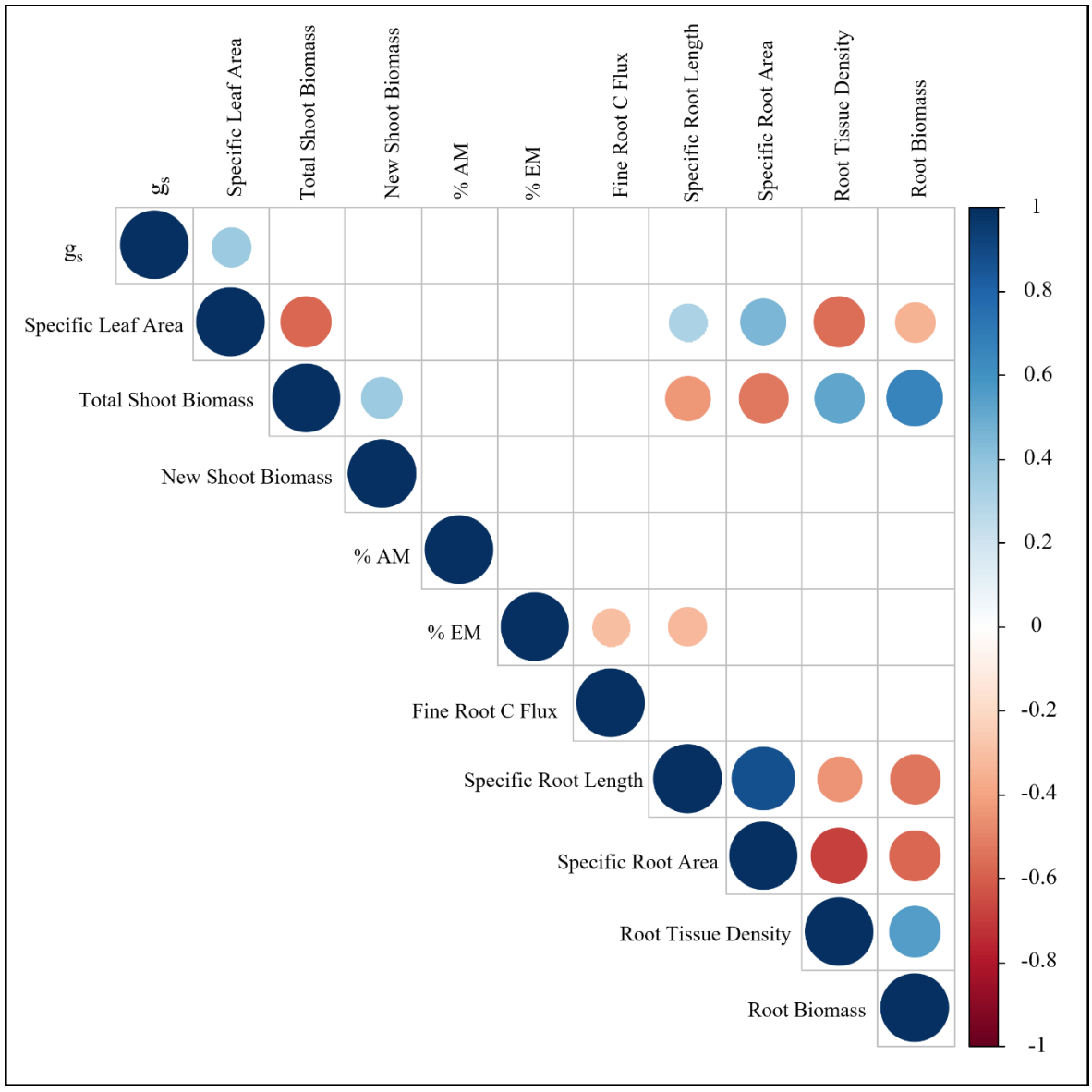

Figure 3. 9. Pearson's correlation matrix of combined aboveground (specific leaf area (m^2 g^{-1}), total shoot biomass (g), new shoot biomass (g), stomatal conductance (g_s (mol H_2O m^{-2} s^{-1})) and belowground traits (root biomass (g), specific root length (m g^{-1}), specific root area(m^2 g^{-1}), fine root C flux (µg C cm^{-1} day^{-1}), mycorrhizal colonization, root tissue density (g cm^{-3}))of *Populus fremontii*. Only significant correlations appear ($\alpha < 0.05$). Color indicates positive (blue) and negative (red) correlations with numbers correlated by side bar ranging from 1 to -1

Tables

Table 3. 1. Mean annual temperature (MAT) (C°) and precipitation (MAP) (mm), with minimum (Min) and maximum (Max) values from source provenances, aggregated by ecotype.

		MAT (C°)			MAP (mm)		
Ecotype	Provenance (n)	Min	Max	Mean	Min	Max	Mean
'Cold & Dry'	2	9.4	9.6	9.5	297	317	307
Cold & Wet'	2	10	10.1	10.05	422	595	508.5
'Warm & Dry'	1	13.2	13.2	13.2	197	197	197
'Warm & Wet '	1	12.4	12.4	12.4	497	497	497

Table 3. 2. Mean (± 1SE) of belowground traits (specific root length (SRL (m g^{-1})), specific root area (SRA (m^2 g^{-1})), fine root C flux (µg C cm^{-1} day^{-1}), root tissue density (RTD (g cm^{-3})) of *Populus fremontii* ecotypes. Significant differences denoted by letters.

	Cold & Dry		**Cold & Wet**		**Warm & Dry**		**Warm & Wet**		**p-value**
SRL (m $g^{-1)}$	61.5	(±9.80)	66.9	(±5.55)	61.9	(±4.22)	59.2	(±4.00)	ns
SRA (m^2 $g^{-1)}$	4.13	(±0.495)	5.10	(±0.399)	4.15	(±0.171)	4.58	(±0.239)	ns
C Flux (µg C cm^{-1} day^{-1})	0.32	(±0.314)	0.0292	(±0.197)	-0.165	(±0.0941)	-0.17	(±0.0605)	ns
RTD (g cm^{-3})	**0.355 c**	**(±0.0271)**	0.219 a	(±0.0164)	0.338 bc	(±0.00849)	0.275 ab	(±0.0149)	> 0.001
Root Biomass (g)	**0.874 b**	**(±0.134)**	0.497 a	(±0.0557)	0.746 ab	(±0.0682)	0.501 ab	(±0.0575)	> 0.01

Table 3. 3. Mean (± 1SE) of aboveground traits (specific leaf area (SLA; $cm^2\ g^{-1}$), diameter at root crown (DRC (mm)), height (cm), new growth (g), and shoot biomass (g)) of *Populus fremontii* ecotypes. Significant differences denoted by letters.

	Cold & Dry	**Cold & Wet**	**Warm & Dry**	**Warm & Wet**	**p-value**
SLA ($cm^2\ g^{-1}$)	82.1 a (±10.5)	**135 b (±7.49)**	82.8 a (±4.32)	**128 b (±7.37)**	>0.0001
DRC (mm)	**5.84 c (±0.322)**	4.9 ab (±0.226)	5.72 bc (±0.233)	4.23a (±0.281)	>0.001
Height (cm)	23.8 (±1.61)	23.9 (±0.881)	25.2 (±1.43)	26.4 (±1.20)	ns
New Growth (g)	0.165 (±0.0965)	0.138 (±0.0604)	0.143 (±0.0727)	0.116 (±0.0561)	ns
Shoot (g)	**3.47 b (±0.980)**	2.16 a (±0.678)	**3.22 b (±0.527)**	1.78 a (±0.713)	>0.0001

Chapter 4 - Synthesis

I aimed to understand the effect of two distinct mycorrhizal types, ecto- (EM) and arbuscular mycorrhizal (AM) fungi, on the roots they inhabit. Through two complementary approaches, including field observations (Chapter 2) and a greenhouse experiment (Chapter 3), this work encompasses a broad range of conditions of the foundation species *Populus fremontii*. As a dual-colonizing species, *P. fremontii* is an ideal model species to isolate the effects of the two mycorrhizal types from host species' identity and environment. In Chapter 2, I used a common garden to address three objectives: 1) confirm dual-mycorrhizal status in *P. fremontii* and investigate colonization rates across a climatic gradient, 2) determine whether fine root C exudation differs between EM and AM-dominated *P. fremontii*, and 3) map root economic traits to understand this species' belowground adaptations to its marginal environment. My results show that both mycorrhizal types colonize *P. fremontii.* However, I found no association between mycorrhizal type and fine root C flux rates and relatively low fine root C flux rates. My results contrast evidence of relatively higher C exudation from fine roots, from previous studies primarily done in temperate and tropical forests (Liese et al., 2018; Meier et al., 2020; Phillips et al., 2011; L. Sun et al., 2021; Yin et al., 2014). The current study also highlighted the high intraspecific variation of root traits along a climate gradient.

In Chapter 3, I attempted to manipulate the dominance of mycorrhizal type through inoculation experiments. However, these initial treatments were ineffective, and all cuttings were colonized by similar ratios of AM: EM fungi. As I found no difference between inoculation treatments across all cuttings, I removed this treatment level and grouped cuttings based on the climates of their source locations climate to create four ecotypes. By grouping cuttings in this way, I explored how source climate characteristics affected *P. fremontii* overall trait plasticity to

explore three objectives: 1) understand if ecotype drives dominate mycorrhizal type, 2) understand if mycorrhizal type in a dual-colonizing species influences root traits across ecotypes, and 3) understand if there is a link between AG-BG traits across ecotypes. My results show that the dominant mycorrhizal type of these cuttings is not correlated with ecotype. Additionally, I show EM colonization relates to morphological and physiological root traits, whereas AM colonization was not correlated with any root trait. Additionally, my results show coordinated AG-BG traits, which vary across ecotypes indicating different growth strategies based on source climate variables.

In short, the dual-colonizing species, *P. fremontii,* consistently associates with both mycorrhizal types simultaneously both in field and greenhouse conditions. The extent to which each mycorrhizal type resides as 'dominant' is variable across source provenances and climatic conditions, however, this work was not able to disentangle these factors. Future work should include overlapping provenances and abiotic conditions to decouple genetic and environmental drivers of dominate mycorrhizal type in this species. Additionally, one consistent morphological root trait was correlated with EM colonization, specific root length (SRL; length per mass unit). Specific root length is often used as an efficiency characteristic, i.e., high SRL indicates low carbon (C) investment resulting in thin roots that can exploit the soil environment better. The negative relationship between EM colonization and SRL may indicate a trade-off in C investment for this species to either "outsource" resource acquisition or "do-it-themselves". Varying patterns of AM colonization with root morphological traits requires careful mycorrhizal manipulations of this type to pin-point interaction in a dual-colonized root system.

Finally, fine root C-flux values remained consistently low in both a controlled greenhouse setting and between two distinct field environments, with inconsistent correlations to

mycorrhizal colonization, indicating that host species identity may be more important to this root physiological trait. To my knowledge, fine-root C flux measurements have never been executed on a dual-colonizing tree species, which resides in an extremely arid environment. Overall low fine-root C flux values in this work deviates from commonly reported values expanding the breadth of C-flux measurements and creating a more robust understanding of a root trait that has received increased attention over the last decade. One limitation of the methodology used in this work is the inability to measure solely root or mycorrhizal fungal C-flux. Due to the intimate nature of the mycorrhizal symbiosis, potential feedbacks between the two partners within my closed system were impossible to understand. To gain a clear understanding of fine root C-flux separated into root and mycorrhizal fungal components will require carefully controlled systems to isolate each partners role.

In all, roots and mycorrhizal fungi have complex interactions that are context-dependent. Here, I examined one species to hold host identity constant which resulted in three common patterns across studies; 1) AM and EM fungi co-occurred on roots of *P. fremontii,* 2) EM colonization interacts with a root efficiency trait (SRL), and 3) this tree species is relatively conservative relative to its fine root C-flux. Root traits including morphological, physiological, and biotic interactions with mycorrhizal fungi are nuanced, as reflected by various inconsistent patterns within this work. These results highlight our limited understanding of these belowground systems and suggest caution should be applied when using mycorrhizal type as a predictor for various ecosystem processes such as C cycling.

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Supplementary Information – Chapter 2

Table S2.1. Analysis of variance on percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)) of *Populus fremontii* among provenances in the Low Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
% AM Colonization					
Provenance	3	505.3	168.4	1.586	0.22
Residuals	24	2549.2	106.2		
% EM Colonization					
Provenance	3	558.5	186.2	3.042	0.048
Residuals	24	1468.7	61.2		

Table S2.2 Analysis of variance on percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)) of *Populus fremontii* among provenances in the High Elevation Garden.

% AM Colonization	df	Sum sq	Mean sq	F - Value	Pr (>F)
Provenance	2	194.4	97.18	1.43	0.26
Residuals	25	1699.4	67.98		
% EM Colonization					
Provenance	2	132.2	66.09	0.637	0.54
Residuals	25	2592	103.68		

Table S2.3. Analysis of variance on fine root carbon flux for *Populus fremontii* among provenances at the Low Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
Provenance	3	0.2424	0.0808	0.824	0.49
Residuals	24	2.3538	0.09808		

Table S2.4 Analysis of variance on fine root carbon flux for *Populus fremontii* among provenances at the High Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
Provenance	2	0.1235	0.06176	0.591	0.56
Residuals	25	2.6108	0.10443		

Table S2.5. Analysis of variance on fine root starch and sugar concentrations (mg g^{-1}) of *Populus fremontii* among provenances at the Low Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
Sugar					
Provenance	3	410.6	136.85	2.233	0.11
Residuals	24	1471	61.29		
Starch					
Provenance	3	931	310.4	1.195	0.33
Residuals	24	8.529	0.3412		

Table S2.6. Analysis of variance on starch and sugar NSC concentrations (mg g^{-1}) of *Populus fremontii* among provenances at the High Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
Sugar					
Provenance	2	48	23.78	0.141	0.87
Residuals	25	4216	168.66		
Starch					
Provenance	2	0.798	0.3989	1.169	0.33
Residuals	25	8.529	0.3412		

Table S2.7. Analysis of variance on total root length, fine root surface area (SA) and total SA of *Populus fremontii* among provenances at the Low Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
Total Root Length					
Provenance	3	2131	710.4	0.37	0.78
Residuals	24	46091	1920.5		
Fine Root SA					
Provenance	3	17.06	5.688	0.593	0.63
Residuals	24	230.26	9.594		
Total SA					
Provenance	3	23.2	7.739	0.424	0.74
Residuals	24	437.6	18.234		

Table S2.8. Analysis of variance on total root length, fine root surface area (SA) and total SA of *Populus fremontii* among provenances at the Low Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
Total Root Length					
Provenance	2	4743	2371	0.654	0.53
Residuals	25	90712	3628		
Fine Root SA					
Provenance	2	36.7	18.37	0.924	0.41
Residuals	25	469.8	19.87		
Total SA					
Provenance	2	51.7	25.84	0.797	0.46
Residuals	25	810.3	32.41		

Table S2.9. Principal component analysis on root morphological and chemical traits of *Populus fremontii* at the Low Elevation Garden. Specific root length (SRL (m g^{-1})), specific root area (SRA (m^2 g^{-1})), fine root C flux (μg C cm^{-1} day^{-1} percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD (g cm^{-3})), non-structural carbohydrate (NSC (g g^{-1})) as sugar and starch.

	Component 1	Component 2
Loadings		
SRL	-0.492	
SRA	-0.524	
C Flux	-0.231	
% AM	0.282	0.354
% EM	0.243	0.199
RTD	0.462	
Sugar		-0.672
Starch	0.263	-0.607
Variance Explained	0.39	0.22

Table S2.10. Principal component analysis on root morphological and chemical traits of *Populus fremontii* at the High Elevation Garden. Specific root length (SRL (m g^{-1})), specific root area (SRA (m^2 g^{-1})), fine root C flux (μg C cm^{-1} day^{-1} percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD (g cm^{-3})), non-structural carbohydrate (NSC (g g^{-1})) as sugar and starch.

	Component 1	Component 2
Loadings		
SRL	0.44	
SRA	0.487	-0.144
C Flux	0.171	-0.600
% AM	0.273	0.606
% EM	0.333	
RTD	-0.363	0.347
Sugar	0.304	0.341
Starch	-0.361	-0.101
Variance Explained	0.37	0.20

Table S2.11. Analysis of variance on diameter at breast height (DBH; cm), height (m), and stomatal conductance (g_s; mol H_20 $m^{-2}s^{-1}$) among provenances of *Populus fremontii* at the Low Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
DBH					
Provenance	3	37.1	12.366	10.65	9.4E-05
Residuals	26	30.19	1.161		
Height					
Provenance	3	21.548	7.183	26.29	4.9E-08
Residuals	26	7.105	0.273		
G_s					
Provenance	3	0.01464	0.00488	9.419	0.00022
Residuals	26	0.01347	0.00052		

Table S2.12 Analysis of variance on diameter at breast height (DBH; cm), height (m), and stomatal conductance (g_s; mol H_2O $m^{-2}s^{-1}$) among provenances of *Populus fremontii* at the High Elevation Garden.

	df	Sum sq	Mean sq	F - Value	Pr (>F)
DBH					
Provenance	2	333.5	166.7	2.477	0.10
Residuals	27	1817.1	67.3		
Height					
Provenance	2	3.34	1.668	1.181	0.32
Residuals	27	38.13	1.412		
g_s					
Provenance	2	0.0028	0.0014	2.154	0.14
Residuals	27	0.01755	0.00065		

Supplementary Information – Chapter 3

Table S3.1. Analysis of variance on percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)) of *Populus fremontii* among inoculation treatments.

	df	Sum Sq	Mean Sq	F value	Pr (>F)
% AM					
Treatment	3	95.2	31.74	1.521	0.22
Residuals	40	834.4	20.86		
% EM					
Treatment	3	797	265.5	0.971	0.42
Residuals	40	10934	273.4		

Table S3.2. Analysis of variance on percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)) of *Populus fremontii* among ecotypes.

	df	Sum Sq	Mean Sq	F value	Pr (>F)
% AM					
Ecotype	3	49.3	16.42	0.746	0.53
Residuals	40	880.3	22.01		
% EM					
Ecotype	3	739	246.3	0.896	0.45
Residuals	40	10992	274.8		

Table S3.3. Analysis of variance on root morphological and physiological traits of *Populus fremontii* cuttings. Specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 g^{-1}), fine root C flux (µg C cm^{-1} day^{-1}), percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD; g cm^{-3}) of *Populus fremontii* among ecotypes.

	Df	Sum Sq	Mean Sq	F value	P Value
SRL (m g^{-1})					
Group	3	415	138.4	0.275	0.843
Residuals	40	20091	502.3		
SRA (m^2 g^{-1})					
Group	3	8.09	2.698	1.523	0.223
Residuals	40	70.87	1.772		
C Flux (µg C cm^{-1} day^{-1})					
Group	3	1.589	0.5296	1.012	0.397
Residuals	40	20.922	0.5231		
RTD (g cm^{-3})					
Group	3	0.1467	0.04889	11.83	1.10E-05
Residuals	40	0.1653	0.00413		
Root Biomass (g)					
Group	3	1.19	0.3966	4.924	0.00527
Residuals	40	3.222	0.0805		

Table S3.4. Principal component analysis on root morphological and physiological traits of *Populus fremontii* cuttings. Specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 g^{-1}), fine root C flux (μg C cm^{-1} day^{-1}), percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD; g cm^{-3}).

	Component 1	Component 2
Loadings		
% AM	0.173	
% EM	0.212	-0.675
C Flux	0.111	0.679
SRL	-0.561	0.103
SRA	-0.608	
RTD	0.478	0.256
Variance Explained	0.42	0.22

Table S5.5. Analysis of variance on fine root C flux (μg C cm^{-1} day^{-1}) of *Populus fremontii* among ecotypes.

	df	Sum Sq	Mean Sq	F value	Pr (>F)
Carbon Flux					
Ecotype	3	1.589	0.5296	1.012	0.40
Residuals	40	20.922	0.5231		

Table S3.6. Principal component analysis on aboveground and belowground traits of *Populus fremontii* cuttings. Specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 g^{-1}), fine root C flux (μg C cm^{-1} day^{-1}), percent mycorrhizal colonization (arbuscular (AM) and ectomycorrhizal (EM)), root tissue density (RTD; g cm^{-3}), specific leaf area (SLA; m^2 g^{-1}), total shoot biomass (g), new shoot biomass (g), stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}).

	Component 1	Component 2
Loadings		
% AM	0.13	0.12
% EM	0.146	-0.553
C Flux		0.63
SRL	-0.383	0.119
SRA	-0.43	0.119
RTD	0.389	
Total Root Biomass	0.394	0.234
g_s	-0.156	-0.196
SLA	0.331	-0.149
Total Shoot Biomass	0.394	-0.112
New Shoot Biomass	0.171	-0.373
Variance Explained	0.37	0.14

Table S3.7. Analysis of variance on specific leaf area (SLA; cm^{-2} g^{-1}), diameter of root crown (DRC; mm), height (cm), new growth (g), and total shoot biomass (g) of *Populus fremontii* among ecotypes.

	df	Sum Sq	Mean Sq	F value	Pr (>F)
SLA					
Ecotype	3	27729	9243	12.08	9.1E-06
Residuals	45	30616	765		
DRC					
Ecotype	3	17.29	5.764	6.567	0.00089
Residuals	45	39.49	0.878		
Height					
Ecotype	3	47.9	15.96	0.855	0.47
Residuals	45	839.8	18.66		
New Growth					
Ecotype	3	0.01257	0.00419	0.814	0.49
Residuals	45	0.02316	0.00515		
Shoot					
Ecotype	3	21.76	7.252	13.35	2.3E-06
Residuals	45	24.45	0.543		

Table S3.8. Analysis of variance on stomatal conductance (g_s (mol H_2O m^{-2} s^{-1}) of *Populus fremontii* among ecotypes.

	df	Sum Sq	Mean Sq	F value	Pr (>F)
g_s					
Climate Group	3	0.01064	0.00355	1.266	0.30
Residuals	40	0.11201	0.0028		

Table S3.9. Analysis of variance on stomatal conductance (g_s (mol H_2O m^{-2} s^{-1}) of time periods (9 AM, 12 PM, 3 PM) between *Populus fremontii*'s ecotypes.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
9:00 AM					
Ecotype	3	0.05803	0.019344	2.678	0.060
Residuals	40	0.28894	0.007223		
12:00 PM					
Ecotype	3	0.00467	0.00156	0.438	0.73
Residuals	40	0.142	0.00355		
3:00 PM					
Ecotype	3	0.00144	0.00048	0.64	0.59
Residuals	40	0.02995	0.00075		

Table S3.10. Analysis of variance on stomatal conductance (g_s (mol H_2O m^{-2} s^{-1}) of *Populus fremontii*'s ecotypes between time periods (9 AM, 12 PM, 3 PM).

	df	Sum Sq	Mean Sq	F value	Pr (>F)
Cold & Dry					
Time	2	0.04746	0.023732	3.841	0.033
Residuals	30	0.18538	0.06179		
Cold & Wet					
Time	2	0.08602	0.04301	10.69	0.00018
Residuals	42	0.16902	0.00402		
Warm & Dry					
Time	2	0.01607	0.08035	6.891	0.0045
Residuals	24	0.02828	0.001178		
Warm & Wet					
Time	2	0.11217	0.06109	18.74	1.3E-05
Residuals	24	0.07822	0.00326		

Table S3.11. Permutational multivariate analysis of variances (PERMANOVA) on aboveground, belowground, and combined traits of *Populus fremontii* four ecotypes.

	Df	Sum Sq	Mean Sq	F Model	R^2	P Value
Aboveground Traits						
Ecotype	3	0.88198	0.29993	9.4642	0.4132	0.001
Residuals	40	1.24254	0.031064		0.5868	
Total	43	2.12452			1.00	
Belowground Traits						
Ecotype	3	0.09984	0.033279	0.8593	0.06055	0.53
Residuals	40	1.54913	0.038728		0.93945	
Total	43	1.64896			1.00	
Combined Traits						
Ecotype	3	0.18158	0.60527	1.6631	0.1109	0.11
Residuals	40	1.45572	0.03693		0.8891	
Total	43	1.6373			1.00	