EARLY TRADE-OFFS OF ROOT TRAITS ON A MYCORRHIZAL COLLABORATION GRADIENT

by
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Aboveground plant traits exist on a spectrum defined by trade-offs between conservative and acquisitive resource uptake. However, belowground traits do not fit this axis because the uptake of soil nutrients can be outsourced to mycorrhizal mutualists. Thus, adding another axis in the root economic space that represents collaboration with arbuscular mycorrhizal (AM) fungi increases our understanding of fundamental variation in root traits. Mature roots exhibit trade-offs between investing in longer root structures that travel farther, or thicker roots that can better support mycorrhizae. Here, I explore whether young roots display trade-offs in collaboration intensity and if so, how long after germination species will begin to reflect niches on this axis. I hypothesize a negative correlation between root growth rate and colonization rate of AM fungi and that the relationship between these variables increases over time as plants refine their resource uptake strategy. I grew five native forb species for twenty-five days and sampled plants at eight time points after germination. Upon sampling, I recorded root length by analyzing the root's structure and then quantified percent colonization by AM fungi. I found that despite variation in species' root length and colonization rates, they did not form a negative relationship. The correlation of length and percent colonization did increase over time with intraspecific variation in the degree and direction of this correlation. The results of this study provide further insight on young root trait variation and benefit our understanding of species interactions in the rhizosphere that contribute to community dynamics.

Introduction

Explaining patterns of variation in plants and their communities has long been a central goal in plant ecology and evolution. Integrating functional trait-based approaches, which connect a plant's morphology to its role in the environment, has proved to be important in achieving this goal. This provides a mechanistic understanding of species-level differences in plants' growth, reproduction, and survival which can be scaled up to explain species assemblages based on factors such as fitness differences or resource partitioning (Kraft et al., 2015; Silvertown, 2004). Because functional traits are valuable for explaining species coexistence, they also increase the predictive success of models proposing how communities will respond to climate changes (Chalmandrier et al., 2022; Laughlin, 2014).

More recently, trait-based approaches have been extended to the rhizosphere to explore how root traits contribute to plant community interactions (Li et al., 2017; McCormack et al., 2017). Patterns in functional trait variation can often be explained by trade-offs that arise when assessing the covariance of traits, thus creating an axis spanning contrasting plant strategies. Trade-offs arranged on this "economic" axis were first applied to aboveground traits spanning from traits that support conservative to acquisitive growth (Wright et al., 2004). But, when root traits were fit to this axis to explain resource uptake strategies, contrasting linear relationships were found (Kong et al., 2019; McCormack & Iversen, 2019). This was suggested to be because roots can uptake resources in different ways, with one of these methods being to outsource the task to mycorrhizal fungi (Weemstra et al., 2016).

Bergmann et al. (2020) propose a novel root economic space with two axes— the conservative-acquisitive spectrum plus another axis, termed the collaboration gradient, spanning from increased length and branching of root architecture to thicker roots that better support mycorrhizal associations. This axis is best fit for plants that associate with arbuscular mycorrhizal (AM) fungi because they form arbuscules in root cortex cells, and a larger root diameter increases the cortex area that is available for AM fungi to colonize (Brundrett & Tedersoo, 2018). Since this addition to the root economic space, studies have been testing the fit of the collaboration gradient and using it to help improve our understanding of plant-soil interactions (Rutten & Allan 2023; Spitzer et al., 2021) and how root traits contribute to ecosystem dynamics (Freschet et al., 2020).

Functional trait studies often overlook young roots, whose traits differ from mature roots in the degree of variation and plasticity they can possess, and likewise the collaboration gradient has yet to be applied to this demographic (Rich et al., 2020; Havrilla et al., 2021). As roots increase in age, their ability to uptake water and nutrients decreases and physical changes to the root include a loss of cortical tissues which has implications for the amount of colonization that they can support (Wells & Eissenstat, 2002). A previous study on seedlings found that their functional traits generally differed from those recorded in mature plants, and further suggested that the relationships between traits were more stable than the traits themselves (Garbowski et al., 2021). Studying the patterns of variation in young roots will provide more information on

this important developmental stage and ultimately how it contributes to plant interactions and responses to the environment.

Here, I assess whether young roots experience the same trade-offs on a collaboration gradient as those reported for mature roots and how this relationship might change throughout early development. I hypothesize that as young roots grow, they exhibit a negative relationship between colonization rate and growth rate representing the trade-offs of the collaboration gradient. Secondly, I hypothesize that the strength of the relationship between length and percent colonization will become greater over time as the plants refine their resource uptake strategy. To test these hypotheses, I performed a greenhouse experiment in which I harvested plant roots of five species at sampling times starting from germination until 25 days of growth to compare their length and percent colonization over time.

Methods

Experimental setup

In a greenhouse, I grew samples alone in clear tubes placed at an angle to promote root growth along the bottom side of the tube. This enabled me to measure and track root length as the plant germinated and grew throughout its sampling period. For each species, I destructively sampled five replicates each at eight sampling times (40 samples per species). The plants in the first sample were harvested on the first day that germination was recorded, with the following samples' growing periods spanning 1, 3, 5, 7, 13, 19, and 25 days. I used five annual forbs native to upland prairies in the Willamette Valley: *Calandrinia ciliata, Clarkia purpurea, Collomia grandiflora, Navarretia intertexta, and Plectritis congesta*. I selected these species because of their co-occurrence in the field and variation in phylogenetic relatedness, an important predictor for differences in germination timing (Barak et al., 2018).

For the soil mix, I used a combination of 45% potting soil, 45% sand, and 10% inoculum from the Friends of Buford Park & Mt. Pisgah native plant nursery, from which the seeds were also sourced from. I chose to collect inoculum at this nursery at various locations that had previously contained native prairie species to ensure that the lab grown plants had access to the species of AMF that they typically associate with in the field.

The use of clear tubes for tracking root growth was followed from the experiment presented in Nguyen et al. (2017). Before filling with the soil mix, I closed off each 30.5 cm long tube with two layers of mesh on one end to promote water uptake and reduce soil loss. I planted three seeds per tube at a shallow depth near an outer edge, except for *P. congesta* for which I planted five due to low germination rates in previous pilot studies. I placed the tubes in bins to keep them at a 30° angle with the seeds positioned at the bottom of the tube opening. This ensured that the roots would proceed to grow along the bottom of the tube and could be measured throughout the experiment by lifting up the tube.

After the tubes were seeded, I randomly placed them in one of 26 bins in a greenhouse. I placed weed cloth over the bins in order to reduce the amount of sunlight that reached the body of the tubes so that weeds would not grow within them. I misted the tube opening two to three times daily to achieve optimal germination conditions and also kept the soil moist from bottom-watering with standing water in the bins.

Measurements:

I checked for germination once every day and measured root length of the samples in the longest-growing sampling group every other day. To harvest a sample, I removed the entire plant from its tube and cleaned it in water with tweezers to remove remaining soil. I cut off any green tissue, considering this above-ground biomass, and stored the roots in individual jars with 80% ethanol at 20 °C to remain until analyzed.

To determine root length, the samples were suspended in water and scanned in a flatbed-scanner at 400 dots per inch. Scans were analyzed using WinRHIZO™ 2021a (Regent Instrument). To enable the staining of AM fungi, I broke down the structural components of the roots by soaking them in 10% KOH for 12 hours or 24 hours depending on the root size, based on results seen in previous trials. I charged them for one minute with 5% HCl before soaking in trypan blue solution (50% glycerol, 47.5% ml distilled water, 2.5% acetic acid, and 0.1 g trypan blue dye per 1 L of solution) for 24 hours to stain any AM structures. I returned the roots to jars and stored them in a destaining solution (50% glycerol) for at least 24 hours to remove any remaining stain on the root structures.

At this point I checked if they were effectively cleared under a light microscope, ensuring that only the AM structures showed up in dark blue dye and that the root was clear. If the root was not clear enough to perform further analysis, I returned it to the destaining solution in order to remove more of the stain and increase contrast between plant tissue and AM fungi. If clear, I proceeded to quantify the degree to which arbuscular mycorrhizae had colonized each root. I cut up the long roots to standardize the root sizes and placed a random sample of these pieces into a petri dish with a grid based on the methods presented by Brundrett (2008). Under a dissecting microscope I recorded whether there was AM fungi on roots intersecting the grid for at least 100 intersections, rearranging the roots after recording everything in the petri dish. I calculated total percent colonization by dividing the amount of root intersections that contained AM fungi by the total amount of intersections recorded.

Statistical Analysis:

All analyses were conducted in RStudio (R Version 4.1.2 (2021-11-01)). To test whether species are investing in increasing their root length or their colonization by AM fungi, I found the average root growth and fungal colonization rates for each species. I calculated the average growth rate for each species by fitting an exponential model with the lengths recorded from each

sample. Average colonization rate was calculated by fitting a logistic regression with the binomial metric of colonization from samples in order to determine the rate at which species become and stay colonized. To evaluate how the relationship between root length and percent colonization changed over time, I calculated the Pearson correlation of these two variables using samples that had at least one replicate with fungi present. I performed a linear regression to estimate the effect of time on the strength of the absolute value of the correlation in order to examine whether the relationship becomes greater over time, regardless of its specific direction. All models were fit with the package brms (Version 0.07.11; Bürkner, 2017).

Results

Species varied in the rate at which their roots grew, notably for *N. intertexta* which had a considerably slower rate of growth than others (Figure 1). *Calindrinia ciliata* had the fastest growth in the first week after germination but then reached stagnation after about 10 days and had a final length at day 25 within the ranges of *C. purpurea* and *P. congesta*.

Plectritis congesta was the only species to consistently associate with AM fungi throughout its growth and a generalized linear model estimated that colonization percent increased 0.94% +/- 0.54% (95% CI) every day (Figure 2). *Calindrinia ciliata, C. grandiflora,* and *N. intertexta* had only one to three individuals that were colonized with AM fungi, with all other harvested individuals being absent of AM fungi. *Collinsia grandiflora* showed relatively early colonization with no evidence of colonization later in life which is likely due to low germination success. Only one species, *C. purpurea,* experienced no colonization throughout the experiment.

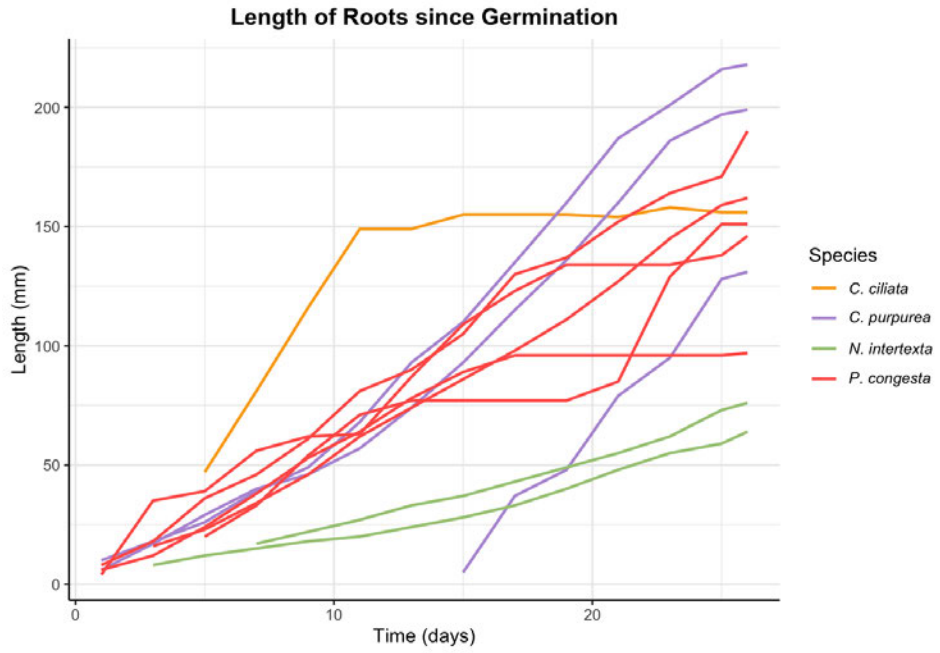

Figure 1. Change in root length over time (days) of the individuals in the longest sampling period. Length of *C. grandiflora* was not obtained because there were no individuals that germinated in that sampling group.

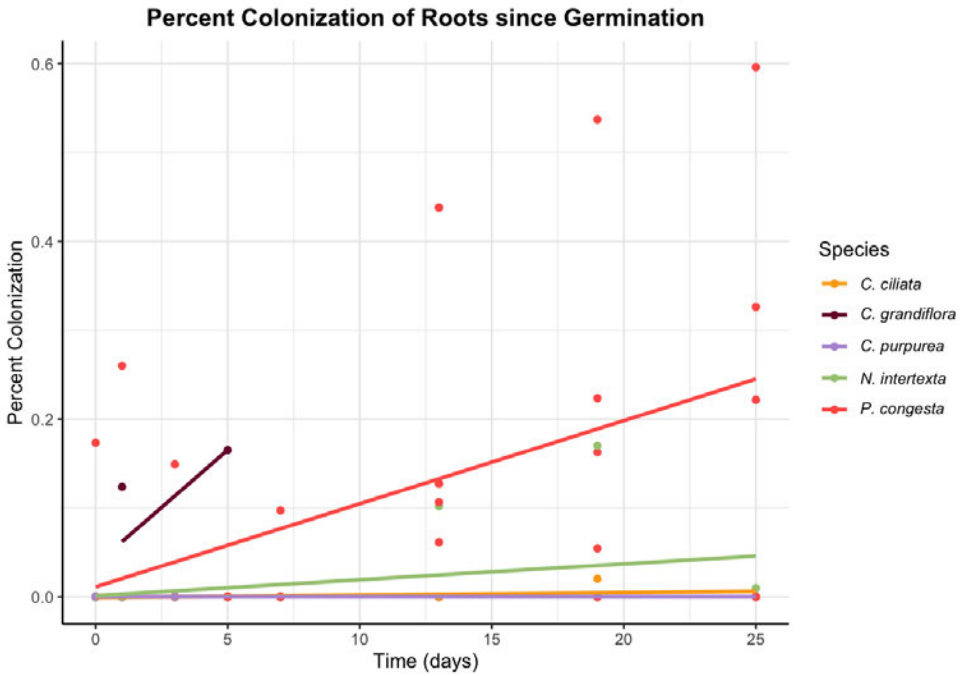

Figure 2. Percent colonization of individuals at the time of harvest, colored by species.

Although species varied in both their root growth rates and rate of colonization by AMF, there was no consistent relationship between these growth rates (Figure 3). Two of the species, *C. purpurea* and *N. intertexta*, had a relatively fast rate in only one of these aspects and not the other. *Plectritis congesta* and *C. ciliata* were able to support fast rates of both growth and colonization.

The magnitude of the correlation of length and colonization increased as the growing period increased (0.0199 +/- 0.0186; Figure 4). The 95% confidence interval of the posterior distribution did not contain zero, indicating a positive relationship of correlation over time. There were differences in the direction of the correlation between length and colonization, and this was not consistent for samples within the same species. The species with the most consistent colonization throughout the experiment, *P. congesta*, experienced the most variation in correlation direction and amount within about the first week before leveling off.

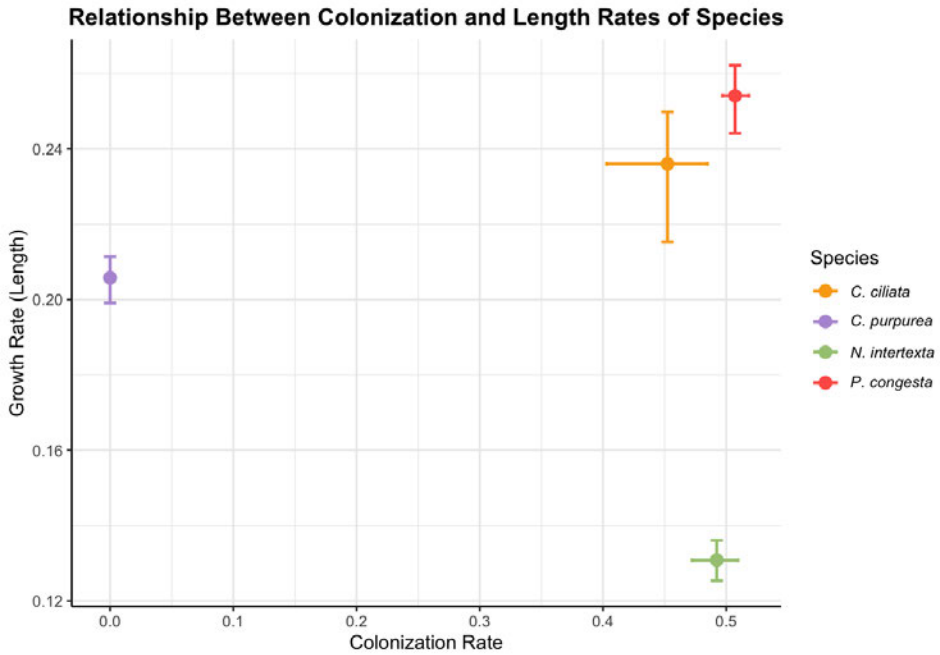

Figure 3. Relationship between the average growth rate of root length and average colonization rate by AM fungi. Coefficients from the logistic models fit for the colonization of each species are plotted against the growth rates from the exponential models of length. The upper and lower limits of the 95% confidence interval for both are plotted. *Collomia grandiflora* was omitted due to its small sample size caused by limited germination success.

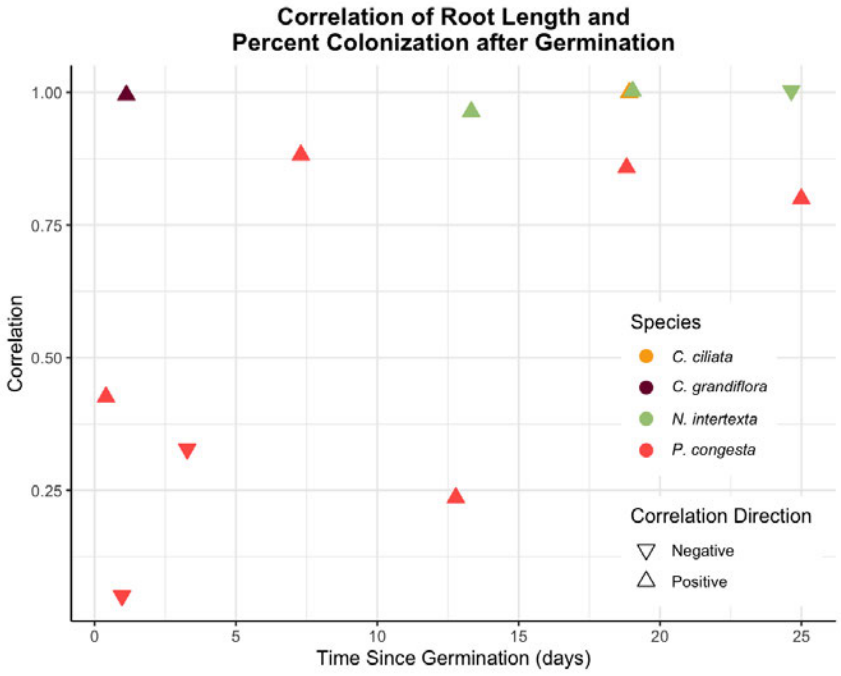

Figure 4. Absolute value of the correlation of root length and percent colonization over time of samples that were colonized with AM fungi. Points are colored by species and arrow directions indicate the direction of the correlation.

Discussion

I hypothesized that young roots exhibit a negative relationship between their AM colonization and growth rates because they experience the same trade-offs on the collaboration gradient that have been recorded in mature plants (Bergmann et al., 2020). I did not find support for this in my analysis, but there was support for interspecific variation which is consistent with previous findings on early root trait variability (Havrilla et al., 2021). Two species, *C. purpurea* and *N. intertexta*, may have experienced trade-offs in collaboration intensity because they had either a fast growth or colonization rate, but did not exhibit both. This did not lead to an overall negative relationship between these rates because the other species, *C. ciliata* and *P. congesta*, were able to support high growth and colonization rates.

The ability of these two species to have relatively high growth and colonization rates may indicate that they have a higher level of fitness. But we know that all of these species co-occur in the field and fill a very similar niche, so there must be other factors that account for these fitness differences. One explanation is that *C. ciliata* and *P. congesta* are experiencing potential trade-offs elsewhere, such as limitations in their aboveground growth. Mature plants experience trade-offs in how they allocate resources for above- or belowground growth that are often context dependent on environmental pressures (Weiner, 2004; Wardle & Peltzer, 2003). Expanding this framework to young plants may help to explain how these species can co-occur despite different rates of belowground development. Additionally, there may be further pressure to reduce fitness differences from resource limitation slowing the speed of fast developing roots, or mediation from the indirect effects of competition with other high fitness plants.

The second part of my hypothesis was that as the roots grew, the relationship between their length and percent colonization would become greater over time. The linear regression of the correlation of these two variables over time resulted in a positive relationship which supports my hypothesis. This indicates that the relationship between root length and percent colonization became stronger as the roots increased in age. Previous research suggests that even though there is more variation in younger roots, the multivariate relationships between their traits are consistent throughout plant development (Garbowski et al., 2021). I instead found that the relationship became greater over early development which may be explained by the difference in relationships from their study compared to mine. Because the collaboration gradient integrates AM fungi which are not inherent from germination like other root traits, it makes more ecological sense for the trait relationship to strengthen over time as AM fungi become more established rather than be unchanging throughout early development.

Species did not always show consistency in the direction of the correlation between root length and percent colonization. Most samples had a positively related correlation of their colonization and length but a quarter of the samples were negative, which was what I had predicted to see for the overall species-level trends. Plants show high intraspecific trait variation in early development, and research suggests that the direction of trait changes is usually consistent within a species and most variation is actually seen in the magnitude of developmental changes (Henn & Damschen, 2021). My study contradicts these findings because I found

variation in intraspecific developmental rates as well as in the direction of the relationship between length and colonization throughout plant growth. This indicates that even within the same species there are differences in the degree to which a plant is facing trade-offs in its resource uptake strategy.

To better understand patterns in functional trait variation, plant ecologists should design experiments with larger temporal scopes and species diversity. I found variation in root length and the degree to which plants utilize AM associations using five annual forbs which fulfill similar roles in their environment. There could be a clearer pattern in this variation that arises when considering broader categories of plants such as different plant habits, lifespans, or environments. In addition, during 25 days of growth *C. purpurea* did not form associations with mycorrhizal fungi, but this does not ensure that it would never form these relationships. A study with a longer growth period may be able to determine with higher confidence that this species does not have a relationship with AM fungi.

There is much more to learn about the variation in young root traits and the degree to which they experience trade-offs on a collaboration gradient. Further study on the patterns of this variation, such as other potential trade-offs, is integral for our understanding of how young roots impact community composition and ecosystem functioning. All plant life stages contribute to a species' population dynamics and thus early life cannot be ignored when studying larger scale patterns in ecology and evolution. In addition, having trait data informs us when developing roots ultimately shift along established trait axes. Measuring changes in functional traits can provide insight into how plants will adjust to situations such as invasive species or climate change, and how this affects community dynamics (Funk et al., 2016; McCormack et al., 2017). However, trait databases use values obtained from mature roots, and this can cause young plants to be overlooked when studying plant responses to change. Placing greater emphasis on young root traits would improve management or restoration practices by increasing our knowledge on how this life stage contributes to plant responses and community dynamics in a changing world.

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