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FINE ROOT RESPIRATION IN QUERCUS RUBRA IS INFLUENCED BY MULTIDIMENSIONAL TRADE-OFFS IN ROOT TRAIT SPACE

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FINE ROOT RESPIRATION IN *QUERCUS RUBRA* IS INFLUENCED BY MULTIDIMENSIONAL TRADE-OFFS IN ROOT TRAIT SPACE

By

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Abstract

Economic theory is often applied to plant strategies, arguing that trade-offs exist between maximizing quick returns on investment within short lifespans or producing longer-lived tissues with slower returns. While these trade-offs are well-studied aboveground, their influence on belowground traits, including root respiration, remains unclear. Resource heterogeneity and symbioses with mycorrhizal fungi may complicate root trade-off axes beyond those predicted by economics theory alone. Investigating fine root trade-offs in *Quercus rubra* populations along a latitudinal gradient in the Midwest, USA, we identified two distinct axes of variation in root traits: one related to a potential "fungal symbiosis" trade-off and another representing a traditional economics trade-off. Root respiration aligned with the rate of return on investment axis, indicating that this carbon flux from terrestrial carbon sinks is influenced by economics strategies. Consequently, strategy shifts across the latitudinal gradient may alter these forests' potential to maintain carbon sink strength as the climate changes.

1 Fine root respiration in *Quercus rubra* is influenced by multidimensional trade-offs in root trait space

1.1 Introduction

Ecologists have long wrestled with how environmental factors shape plant strategies, arguing the relative importance of stress, competition, disturbance, and nutrient availability. Economic theory was first applied to plant responses to resource availability in 1985 (Bloom et al. 1985). These authors argued that plants must have both genetic long-term resource acquisition strategies based on the environments they evolved in and short-term, plastic strategies to respond to dynamic conditions (Bloom et al. 1985). Out of this context arose papers hypothesizing that plant traits vary in response to past and current conditions along trade-off axes described in the leaf (Wright et al. 2004), wood (Chave et al. 2009), and root economics spectra (Comas et al. 2002, Roumet et al. 2006), as well as coordination between these above- and belowground strategies (Reich et al. 1998, Valverde-Barrantes et al. 2015).While the leaf economics spectrum is now well-supported, belowground traits have only recently been investigated in the context of economic theory.

The root economics spectrum theory ties together demographic factors of roots into two groups of commonly associated traits. Where resource conservation is prioritized, roots are expected to be denser and thicker, with lower metabolic activity and longer lifespans. On the other side of the spectrum, where individuals prioritize acquisition, roots ought to be less dense, longer, thinner, and more metabolically active (Fig. 1.1). The theorized spectrum applies only to absorptive fine roots, typically in the top 10 cm of soil and defined as one of the first three root orders or less than 0.5 to 2 mm in diameter (Wang et al. 2019). The acquisitive type of fine roots cost less to build per unit length or surface area, making them easy to senesce if maintenance costs begin to outweigh resources and cheap to produce relatively quickly in response to resource variation within the soil. On the other hand, thicker dense roots have a greater upfront cost and slower return on investment, but are associated with lower maintenance needs and thus longer lifespans (McCormack et al. 2012, Adams et al. 2013). This leads to the prediction that on the acquisitive end of the spectrum, root nitrogen (N; mg g^{-1}) and specific root length (SRL; m g^{-1}) will be greater, whereas fine roots on the conservative end of the spectrum will show greater values of tissue density (RTD; g cm^{-3}) and average diameter (RD; mm).

The root economics spectrum is generally focused on comparisons of root traits across many species. To our knowledge, only two studies have investigated the root economics spectrum within a single species (Hajek et al. 2013, Isaac et al. 2017). Intraspecific trait variation, relative to a species' average rather than global variation, may have different controls than that between species, and the range of variability possible within species and populations may determine where they can survive and whether they will be able to adapt to changing conditions. Both individual acclimation and genetic local adaptations are possible, and may be related to changes in nutrient availability, soil type, or temperature and precipitation regimes. Studies investigating one species and broader studies that have incorporated intraspecific analyses have found substantial variation when compared to interspecific differences as well as complex environment-trait interactions across differences in soils, temperature, precipitation, and management practices (Comas and Eissenstat 2009, Hajek et al. 2013, Isaac et al. 2017, Paradiso et al. 2019). In response to this need, we have focused this study within the Midwestern range of *Quercus rubra* in the U.S. Additionally, few studies have integrated measurements of fine root respiration with the root economics spectrum, which we incorporated here.

Root respiration rates are higher when roots are expending more energy for nutrient acquisition, leading to the expectation that specific fine root respiration rates (i.e., respiration rates per unit root mass, RSR; nmol CO_2 g^{-1} s^{-1}) should be greater in more acquisitive roots. Root N is often a strong predictor of RSR because more proteins involved in respiration for nutrient uptake and transport result in roots with higher N concentration (Zogg et al. 1996, Burton et al. 2002). For example, root N explained 88 % of the variation in fine root respiration rates in larch and ash trees in a planted stand (Jia et al. 2013). Root N can reflect an acquisitive strategy or soil N availability (Zhao et al. 2022), but either of these effects may result in the same relationships with respiration because the root economics theory anticipates acquisitive strategies in environments with greater resource availability. Positive correlations between SRL and RSR may also be explained by greater nutrient uptake and soil exploration (Paradiso et al. 2019). On the conservative end of the spectrum, higher values of both RTD and RD maximize longevity and nutrient storage, resulting in decreased cellular activity and maintenance respiration rates (Makita et al. 2009, Miyatani et al. 2018, Han and Zhu 2021). Two global meta-analyses have found that RSR, as anticipated, tended to be positively correlated with root N and SRL and negatively correlated with RTD (Han and Zhu 2021, Gao et al. 2021).

This provides some support for the root economics spectrum, demonstrating that fine roots with higher RSR, presumably to maximize resource uptake, tend to be longer and thinner with higher N concentration and lower density.

Despite these results supporting the root economics spectrum, there are other authors that call its existence into question or seek to expand it. For example, some studies across species have found only weak relationships of SRL and RD with RSR while others have found a negative correlation between RTD and RD or a lack of correlation between root N and SRL (Valverde-Barrantes et al. 2015, Ma et al. 2018, Kong et al. 2019, Han and Zhu 2021). Principal component analyses tend to reveal not one, but multiple axes of root trait variation, with a primary axis reflecting a trade-off between SRL and RD, and a secondary axis representing a trade-off between root N and RTD (Comas and Eissenstat 2009, Liese et al. 2017, Ma et al. 2018, McCormack and Iversen 2019, Paradiso et al. 2019, Han and Zhu 2021, Li et al. 2021). For this reason, the "spectrum" has been expanded into a multidimensional trait "space" (Bergmann et al. 2020). Root respiration tends to correlate with root N in the second axis (Han and Zhu 2021, Gao et al. 2021). A belowground trait space may not perfectly mirror the aboveground economic theory, as was originally proposed, firstly because of the greater heterogeneity of soil resource availability compared to light and CO_2 in the canopy (Valverde-Barrantes et al. 2015, Liese et al. 2017, Paradiso et al. 2019), and secondly as a result of differences in reliance on diverse mycorrhizal communities to aid resource acquisition by the root system (Ma et al. 2018, McCormack and Iversen 2019, Paradiso et al. 2019).

Mycorrhizal dependence is especially complicating, as it provides a pathway by which roots can display an apparently conservative root strategy but still have higher rates of nutrient uptake from their fungal symbionts. Increased mycorrhizal colonization usually covaries with RD and against SRL (Ma et al. 2018, McCormack and Iversen 2019, Paradiso et al. 2019). Trees with a greater degree of mycorrhizal colonization can invest less in elongating their own root systems to increase their belowground zone of influence. For example, the negative correlation between RTD and RD found in multiple studies listed above could be explained by a proposed second dimension of the root economics space describing mycorrhizal colonization. This "fungal collaboration" axis may include an alternative acquisitive suite of trait values, characterized by thicker fine roots and more reliance on mycorrhizal partners. In ectomycorrhizal species, this may result in many root tips to promote mycorrhizal colonization and shifts in carbon investment from their own root length to fungal hyphae for soil exploration (Ma et al. 2018, McCormack and Iversen 2019, Paradiso et al. 2019, Bergmann et al. 2020, An et al. 2022). For ectomycorrhizal species like northern red oak (*Quercus rubra*) where symbionts colonize root tips (Comas et al. 2014, Liese et al. 2017), branching intensity, the number of fine root tips per unit root length (BI; tips cm^{-1}), could indicate investment in mycorrhizal associations and may help determine when nutrient foraging strategies are strongly affected by fungal partners.

Quercus rubra naturally inhabits much of the eastern U.S. (Sanders 1990) and is both economically and ecologically significant. *Quercus rubra* affects nutrient cycling through its recalcitrant litter and associations with ectomycorrhizal fungi, hydrology from deep rooting and hydraulic lift, and other trophic levels through the production of acorns and secondary compounds (Cavender-Bares 2019). Red oaks (*Quercus* section *Lobatae*) are important in domestic markets and abroad, retaining their spot as the top U.S. hardwood lumber export group from 1990 to 2020 (Bumgardner 2019). Furthermore, many *Quercus* species studied across lineages have exhibited substantial genetic variation and plasticity within local populations, implying that local adaptation is not the strongest driver of their persistence (Cavender-Bares 2019). Many *Quercus* species have also displayed adaptive plasticity in response to climate change (Lo Gullo and Salleo 1993, Cavender-Bares et al. 2007), and this capacity for individual adjustment indicates that they may display a wide array of trait strategies in response to different environmental conditions.

In this study, we investigated variation in surface fine root traits across the Midwest range of *Q. rubra* in the U.S. to establish whether these traits vary according to a uni- or multidimensional root economics space theory and understand how they influence fine root respiration rates. We hypothesized that (H1), morphological trait trade-offs will be multidimensional, with one axis describing more conservative roots with high RTD and the other describing a fungal collaboration trade-off with SRL at one end and RD and BI at the other. We also hypothesized that (H2), since RSR and N reflect work being done, populations with higher values of these traits will fall on the acquisitive end of the root economics axis, opposite to RTD on the conservative end.

1.2 Methods

1.2.1 Study sites

Quercus rubra fine roots were sampled from 14 sites across the Midwest with mean annual temperatures (MAT) ranging from 5 to 14 °C (Table 1.1; Fig. 1.2). Sites generally represented mature, mixed deciduous stands with a significant component of *Q. rubra* in all stands, and *Q. rubra* dominating the basal area of several (HWUP, NOWI, NOLP; Table 1.1). Sites were visited twice in the summer of 2022, once between June 1st and June 21st, and once between July 5th and July 21st.

1.2.2 Field sampling

At each site, 6 plot centers were set up along a 200 m transect, approximately 40 m apart and near the base of a cluster of 2 to 3 large *Q. rubra* individuals. If no *Q. rubra* were near an intended location, locations were adjusted to the nearest cluster. Forest composition data was collected from each plot center using variable radius sampling with a basal area factor 10 angle gauge and no diameter limit cutoff. Two 6 cm deep soil cores were taken 1 m apart at each plot center using a 10 cm tall by 5 cm diameter core. Soil and root samples were removed from the two cores and combined for a plot-level sample. From these soil samples, live red oak fine roots ≤ 1 mm in diameter were separated based on color, branching, and brittleness, and lightly brushed clean of soil and adhering organic matter for respiration measurements. Approximately 2 g wet weight samples were used, and dry weights ranged from 0.0495 to 0.628 g. Roots were gathered as quickly as possible to keep overall time between excision and respiration measurements below 45 minutes.

1.2.3 Respiration measurements

Fine roots were bundled in a lint-free tissue and placed in a custom cuvette with a 5 cm tall by 5 cm diameter inner chamber (Burton et al. 2012) connected to an infrared gas analyzer in an open system configuration (LI-6800, LI-COR Biosciences, Lincoln, NE). Input carbon dioxide (CO_2) was set at 1000 µL L^{-1} to mimic surface soil CO_2 concentration in a northern hardwood forest, and fan speed was 10,000 rpm. Relative humidity was kept below 60 % to prevent condensation in the chamber. A water bath was used to keep the cuvette at a temperature of 18 °C. Respiration rate was measured as rate of CO_2 evolution per second (µmol CO_2 s^{-1}) to be later normalized by sample dry mass. After measurement, fine root samples were kept on ice and then frozen until later lab analysis.

In July, additional data was logged with an empty cuvette before each sample was measured. These "blank" values were then subtracted from fine root respiration measurements to correct for instrument effects. Blank logs ranged from -0.2 to +1.7 µmol CO_2 s^{-1} compared to our raw respiration measurements of -0.04 to -2.9 µmol CO_2 s^{-1}, where negative values represent the increase of CO_2 in the chamber from respiration (i.e. negative photosynthesis, relative to the instruments normal mode). Subtracting the instrument noise from respiration rates from our relatively small fine root samples can have a measurable effect on the final rates calculated. Blank logs were not taken in June, so the linear relationship between CO_2 match values automatically logged by the infrared gas analyzer and the blank logs in July (P=0.026) was used to predict blank logs from the CO_2 match values in June. These match values are related to measurements taken when the system is run with an empty cuvette because they indicate the correction factor needed to equilibrate the CO_2 concentration readings between the sample and reference gas analyzers when they are receiving the same air stream, the magnitude of which could be related to small leaks, time, or temperature.

Respiration rate measurements, following adjustment with blank logs, were divided by the total fine root dry weight of each respiration-only sample to calculate root specific respiration rates at 18 °C (RSR; nmol CO_2 g^{-1} s^{-1}) for each plot within a given site.

1.2.4 Sample preparation

In the lab, remaining fine roots were sieved out of each soil sample using 6.3 mm followed by 2.0 mm opening sieves. All other *Q. rubra* fine roots were removed for addition to fine roots hand-sorted in the field. Throughout the remainder of the paper, this full sample will be called the “whole-core sample” while the subsample used for fine root respiration measurements in the field will be called the “respiration-only sample”. Respiration-only samples tended to be made up of longer, thicker, and more branched fine roots, presumably because these are more noticeable in the soil cores and removed preferentially under the time constraint of respiration measurement. All fine root samples were cleansed in deionized water with organic residue removed caught on a 177.8 µm opening sieve. Organic matter caught by the sieve was dried at 65 °C for 24 h and weighed. The percent of fine roots in this organic matter residue from each sample, compared to soil and other organic debris, was visually assessed and multiplied by the organic matter dry weight so that these extra root weights could be added to the total dry weights of samples, obtained below (Burton et al. 2012). These additions made up only ~1.9 % of the final root dry weights on average.

1.2.5 Fine root traits

Cleansed fine roots from the respiration-only samples were spread in a 20 x 30 cm clear scanning tray (Scanning Tray, Regent Instruments, Canada) in approximately 400 mL of deionized water. Samples were scanned on a large flatbed scanner (Expression 10000XL, Epson, Los Alamitos, CA) at 1200 dpi. Images were loaded into RhizoVision Explorer v.2.0.3 (Seethepalli, Anand and York, Larry M. 2020) and analyzed with image thresholding of 110, non-root objects under 0.2 mm filtered out, and root pruning enabled with a threshold of 4 pixels. Total root length, average diameter, total volume, and number of tips were extracted from the image analysis. This process was repeated using the whole-core samples as well in an attempt to obtain morphological trait values with less bias compared to roots hand-picked in the field for the respiration-only samples.

After scanning for morphological traits, fine root samples were oven dried at 65 °C for 48 h. Dry weights of each respiration-only and whole-core sample were obtained using a precision balance (Pioneer Precision PX323, OHAUS, Parsippany, NJ). Specific root length (SRL; m g^{-1}) was calculated as total fine root length divided by the dry mass of scanned roots. Root tissue density (RTD; g cm^{-3}) was the measure of scanned root dry mass divided by its total volume, and branching intensity (BI; tips cm^{-1}) was the number of root tips divided by total root length. The extracted average root diameter (RD; mm) from image analysis was used for each sample. Lastly, all respiration-only samples were ground in a ball mill (8000M Mixer/Mill, Spex SamplePrep LLC, Metuchen, NJ) and 4 mg of each were weighed for mass-based root nitrogen (N; mg g^{-1}) analyses in an elemental analyzer (Carlo Erba NA 1500 NC, CE Elantech).

1.2.6 Statistical analysis

All analyses and visualization were performed in R version 4.1.2 (R Core Team 2021), with tidying using the package *dplyr* (Wickham et al. 2023), and graphics produced using *ggplot2* (Wickham 2009). Data were explored for normality and outliers using histograms and Shapiro-Wilks tests. The distribution of SRL was skewed right and exhibited non-linear relationships with other variables, so a natural log transformation was used to achieve normality.

To test H1, that *Q. rubra* fine root morphological traits follow multidimensional trade-offs, relationships among SRL, BI, RD, and RTD from the whole-core dataset were analyzed by a Pearson's correlation matrix with campaign month, June or July, as the categorical factor. Then, principal component analysis (PCA) was used to identify primary trade-off axes. The function *ggpairs* in the package GGally (Schloerke et al. 2021) was used for the correlation matrix and the *prcomp* function in the *stats* package included in R was used for the PCA.

To test H2, that RSR and root N would align on a root economics axis, root N and all morphological traits listed above from the respiration-only dataset were analyzed with a separate correlation matrix. These traits were then tested for relationships with specific respiration rate using ANCOVAs, with campaign month, June or July, as the categorical factor. Relationships with respiration were then modeled with linear regressions, with one model per campaign month if the interaction between month and the explanatory variable was significant, or one model with both months combined if not. Models were validated using the package *performance* (Lüdecke et al. 2021) to compare residuals with the fitted values, checked for outliers with Cook's distance, and assessed for normality of residuals using Q-Q plots. Lastly, to visualize all axes in the respiration-only dataset and test whether economics and fungal collaboration trade-offs drove trait variation, PCA was used on all traits and respiration rates.

1.3 Results

1.3.1 Morphological trait trade-offs

Quercus rubra fine root morphological traits displayed substantial within-species variation, though only some traits covaried according to the root economics spectrum (H1). In the whole-core dataset, *Q. rubra* fine roots across both months were found to have an average SRL of 52.4 ± 3.98 m g^{-1} (95% CI) and vary from 11.7 to 95.6 m g^{-1}, while RTD averaged 0.231 ± 0.012 g cm^{-3} and varied from 0.101 to 0.482 g cm^{-3}, RD averaged 0.273 mm ± 0.003 and varied from 0.191 to 0.344 mm, and BI averaged 1.09 ± 0.059 tips cm^{-1} and varied from 0.271 to 1.90 tips cm^{-1}.

All correlations between morphological traits from the Pearson's correlation matrix conducted on the whole-core dataset were significant, and unexpectedly, almost all were negative (Fig. 1.3). The strongest three correlations were negative relationships between BI and SRL as well as RTD and SRL, and a positive relationship between BI and RTD (all $P<0.001$; $R^2=0.46$; $R^2=0.68$; $R^2=0.66$, respectively). The other three relationships, RD against SRL ($P=0.002$; $R^2=0.05$), BI ($P<0.001$; $R^2=0.10$), and RTD ($P<0.001$; $R^2=0.08$), were negative and much weaker.

Principal component analysis on the whole-core dataset revealed two primary axes of morphological trade-offs (Table 1.3; Fig. 1.6a). The first principal component (PC1), explaining 64.38 % of variation in the data, described a trade-off between longer, thinner, more branched roots and those with higher tissue density. PC2 explained 29.5 % of the variation and was composed of a trade-off between samples with longer, thinner roots and those with higher average diameter, and PC3 only explained 5.25 % of the variation in a trade-off between more root tips per unit length and higher root tissue density.

1.3.2 Root nitrogen and specific respiration

Both root N and RSR from the respiration-only dataset followed more of the expected relationships with the morphological traits described in H2, that these two traits would be higher in an acquisitive strategy on the economics axis. Across all sites and both months, *Q. rubra* fine roots exhibited an average root N of 7.90 ± 0.193 mg g^{-1} (95 % CI) that ranged from 5.50 to 11.9 mg g^{-1} and RSR of 5.51 ± 0.450 nmol CO_2 g^{-1} s^{-1} ranging from 0.441 to 17.4 nmol CO_2 g^{-1} s^{-1}.

The Pearson's correlation matrix conducted on the respiration-only samples revealed similar relationships to those in the whole-core dataset with two exceptions (Fig. 1.4). As before, a strong negative correlation existed between SRL and RTD ($P<0.001$; $R^2=0.33$). However, the positive relationship between BI and RTD became much weaker ($P=0.034$; $R^2=0.03$), and even more notable, the relationship between BI and SRL reversed to a positive, albeit weak, correlation ($P=0.003$; $R^2=0.05$). Additionally, two previously weak negative relationships, between RD and SRL, and RD and BI, became stronger (both $P<0.001$; $R^2=0.34$ and $R^2=0.22$, respectively). The last negative correlation, between RD and RTD, remained relatively weak ($P<0.001$; $R^2=0.09$).

Correlations with root N tended to align most along an economics axis with SRL at one end and RTD on the other (Fig. 1.4). Root N was positively correlated with SRL ($P<0.001$; $R^2=0.17$) and only marginally with BI ($P=0.054$; $R^2=0.02$). By contrast, N was negatively related to RTD ($P<0.001$; $R^2=0.25$) but showed no relationship with RD.

Root specific respiration rates were positively correlated with traits assumed to be acquisitive when high and negatively with those on the conservative end, with the exceptions of BI and RD. On the acquisitive end, RSR was positively correlated with SRL and root N ($P<0.001$, $P<0.001$, respectively; Table 1.2; Fig. 1.5a, c). Root N exhibited a significant interactive effect with campaign month for RSR, with a steeper slope in June. Conversely, RTD showed the expected negative relationship with RSR while RD displayed a positive, though weak, relationship ($P<0.001$, $P=0.035$, respectively; Table 1.2; Fig. 1.5d, e). Branching intensity was not related to RSR. One outlier in RSR was removed as it was almost double the second highest value (30.29

compared to 17.28 nmol CO_2 g^{-1} s^{-1}) and was from an exceptionally small root sample (0.04 g), making this rate highly unlikely and more subject to error due to the large signal to noise ratio that exists using our method when measuring low absolute CO_2 fluxes generated by very small samples.

Principal component analysis on the respiration-only dataset fit both N and RSR into the first axis with SRL and opposite to RTD. Three principal components together explained 81.45 % of trait variation in the dataset (Table 1.4). The first, explaining 37.37 % of variation in the data, was dominated by the economics trade-off mentioned above, with RTD at one end and N, RSR, and SRL on the other (Fig. 1.6b). The second described a further 30.34 % of the variation, primarily described by variation in BI alone, and PC3 explained 13.74 % of the variation and revealed SRL as opposite to RSR and BI.

1.4 Discussion

1.4.1 Trait ranges

These results show that *Q. rubra* roots occur within a narrow but meaningful range of variation compared to the global spread of traits. Trait averages found here in the whole-core dataset were comparable to those found for *Q. rubra* in a study of 25 mature temperate trees in Pennsylvania, though they tended to be slightly higher with the exceptions of BI and root N from the respiration-only data, both of which were lower (1.09 in our study compared to 3.01 tips cm^{-1}; 7.90 compared to 13.2 mg g^{-1}) (Comas and Eissenstat 2009). Differences could be the result of different sampling methods (tracing the roots in their study and using the first two root orders compared to soil cores with a 1 mm diameter cutoff in this one), different root imaging software, as well as environmental conditions in Pennsylvania compared to our range of sites in the Midwest. Comparatively, a recent meta-analysis found that woody plants can vary from 0.1 to 1.3 g cm^{-3} in RTD and under 50 to as high as 500 m g^{-1} in SRL for a rare few species (Ma et al. 2018). *Quercus rubra* therefore tends to occupy the moderate to high end of SRL and low end of RTD compared to the majority of woody species and, compared only to the temperate species studied in Pennsylvania, tends to have longer, thinner, less dense fine roots than others but with relatively low N concentration and moderate branching intensity. Despite the smaller range of variation offered by a single species, we contend that across the latitudinal gradient there are enough plastically or locally adapted differences in trait strategies to explore how well the root economics spectrum or multidimensional space theories can explain trade-offs in root morphology and physiology. To illustrate this point, the range of variation in RTD found in *Q. rubra* in this study occupies almost half of the total range of variation found for woody species in Ma et al. (2018) and SRL covers about a fifth.

1.4.2 Morphological trade-offs

Results from this study tend to corroborate the idea of the root economics spectrum within *Q. rubra* when looking only at individual correlations between traits from the whole-core samples, with some unexpected results. Morphological traits that are expected to be higher in an acquisitive nutrient uptake strategy according to the root economics spectrum include SRL and BI. Specific root length varied oppositely with RTD and displayed a weaker negative relationship with RD, as expected (Fig. 1.3). For these cases, fine root strategies tended to organize along an axis that prioritizes nutrient acquisition on one end and conservation on the other. However, other trait correlations with RD and BI complicated the results. Economics theory assumes one axis of variation with RTD and RD on the conservative end; in this study, we found that although they both tended to be negatively correlated with traits on the acquisitive end, root tissue density and diameter were also negatively correlated with each other.

Branching intensity was expected to reflect either mycorrhizal dependence, fine root economics strategy, or a combination of both. If it reflected mycorrhizal dependence in the Midwestern *Q. rubra* studied here, we would have expected a positive correlation with RD and negative correlation with SRL. If instead it was more representative of an economic trade-off, we would have expected the opposite, with correlations according to the acquisitive and conservative ends of the spectrum. In contrast, we found that in the whole-core dataset, BI was negatively related to RD and SRL, and positively related to RTD (Fig. 1.3). The first PCA axis, with the most explanatory power (64.38 %), similarly described a trade-off between long, thin roots and those that are denser and have many root tips (Table 1.3; Fig. 1.6a). Positive relationships with RTD, associated in other studies with fine root lifespan (e.g. Bergmann et al. 2020), could suggest that some longer-lived fine roots may also display a high degree of stable mycorrhizal associations. Alternatively, fungal mass on colonized fine root tips could result in higher tissue density than the secondary fine roots accounting for most root length, driving up sample RTD when BI is high.

The negative relationship between RD and BI suggests that mycorrhizal dependence was either not related to BI in *Q. rubra*, unlikely given that root tips are the site of ectomycorrhizal colonization, or not related to RD. Correlations with RD were difficult to assess in this study due to the small range of diameters sampled ($\leq$ 1 mm) so it is possible that sampling from a wider set of diameters or using root orders to define fine roots would have led to a different result. However, others have found similar relationships between BI and RD across temperate tree species as well as between RD and RTD (Comas and Eissenstat 2009, Valverde-Barrantes et al. 2015, Ma et al. 2018). Studies that have found relationships between RD and mycorrhizal colonization tend to be global meta-analyses that span a variety of plant growth forms (e.g., Ma et al. 2018, McCormack and Iversen 2019). Average diameter is relatively phylogenetically conserved relative to other root traits (Valverde-Barrantes et al. 2017, Weigelt et al. 2021, Yan et al. 2022), and its relationship with mycorrhizal reliance is often assumed to be a compensatory mechanism to increase nutrient uptake in more basal lineages with higher RD. These lineages are often associated with the older arbuscular mycorrhizal symbiosis (Wang and Qiu 2006), which relies on increased cortical space for colonization rather than root tips, as in the ectomycorrhizal fungi that associate with *Q. rubra* (Bergmann et al. 2020). If phylogenetic conservation and increased cortical space for arbuscular colonization are the primary causes for increased mycorrhizal dependence with higher RD, then we may not see this relationship within a single ectomycorrhizal species, as we studied here. Rather, BI might be a better indicator of mycorrhizal dependence, as suggested by Yan et al. (2022).

The second PCA axis, explaining less than a third of variation in the data, described a trade-off between specific root length and root diameter. This trade-off aligns with the fungal collaboration trade-off that has been proposed to explain the results of many across-species studies (e.g. Ma et al. 2018, McCormack and Iversen 2019, Paradiso et al. 2019, Bergmann et al. 2020). However, within one species, this trade-off may be more related to heterogeneity of soil resources. These results imply that trees, independently of the PC1 trade-off between SRL, BI, and RTD, have some ability to vary RD. Average root diameter, shown by others to be related to fine root lifespan including in *Q. rubra* (McCormack et al. 2012), may be adjusted depending on the longevity of nutrient patches in the soil, where fine roots may thicken if patches persist. For example, ectomycorrhizal species in the genus *Pinus* are relatively thick-rooted and tend to show less foraging precision than thinner-rooted ectomycorrhizal species, assumed to be an adaptation to environments with evenly distributed soil nutrients (Chen et al. 2016). However, it is not yet clear whether RD varies in response to nutrient stability within populations of a single species, or if there is another explanation for this trade-off.

1.4.3 Root nitrogen and specific respiration

From the respiration-only dataset, trait relationships with root N and RSR more consistently aligned with a root economics spectrum trade-off. As root N is typically understood to reflect enzymatic capacity, high values are predicted to represent a quick return on investment strategy, in which fine roots are relatively inexpensive to build, exhibit a fast metabolism and high nutrient uptake rates, and have short lifespans (i.e., turn over quickly). Here, we found that root N was positively correlated with SRL and BI

(Fig. 1.4), demonstrating that fine roots with a greater investment in length growth and possibly soil exploration also have more enzymatic capacity. On the conservative end of the spectrum, root N was low when RTD was high, showing that fine roots with a higher construction cost likely have a lower metabolism and thus maintenance respiration.

Similar relationships were found for RSR, indicating that even within one species, fine roots on the acquisitive side of the spectrum respired more and displayed other traits associated with low upfront costs. Specific root length is thought to reflect nutrient uptake potential of fine roots, as well as decreased ability to cope with stress (McCormack et al. 2012), and so is typically greater in highly active roots as was shown in our data. On the other hand, RTD was negatively correlated with RSR, which agrees with prior research examining across-species variation (Gao et al. 2021) and indicates that fine roots with higher tissue density have greater storage capacity, but are less active in nutrient absorption. According to the economics spectrum, thicker roots are expected to reflect a more conservative strategy and exhibit lower respiration rates. However, we found that RSR increased by 1.35 nmol CO_2 g^{-1} s^{-1} for every 0.1 mm increase in RD (Fig. 1.4d). If wider fine roots are more colonized by mycorrhizal fungi, higher respiration rates could be the result of increased maintenance respiration for repair and replacement of the costly chitin-rich cell walls in colonizing fungi (Trocha et al. 2010). However, this relationship is weak ($R^2=0.027$), and the majority of fine root RD varied between about 0.3 to 0.4 mm, making it unlikely that RD was a strong driver of fine root respiration.

With RSR and N included, PCA revealed three main axes of variation from the respiration-only dataset, of which the first two explained two-thirds of the variation in the data (66.81 %; Table 1.4 and Fig. 1.6b). These reveal that within *Q. rubra*, the most important fine root trade-offs fall on one axis with RSR, SRL, and N on one end and RTD on the other, and another determined primarily by variation in BI. The first axis may be the result of a root economics trade-off between building denser fine roots with a slower return on investment or constructing a fine root system built for rapid nutrient uptake with high N concentrations, respiration rates, and long, thin roots. This trade-off has been correlated with differences in fine root lifespan both within and across temperate tree species including *Q. rubra*, with a conservative strategy favoring longer lifespan and vice versa for an acquisitive strategy (McCormack et al. 2012, Adams et al. 2013).

Across the range of a species, where populations or individuals fall on this axis may be determined by both the patchiness and supply rates of soil resources. Economics spectrum theory predicts that with high and constant resource supply, strategies will be more acquisitive because there are consistently enough materials and return on investment to keep producing inexpensive, rapidly metabolizing plant tissues. Belowground, however, patchiness may be more important as resources are much less evenly distributed than in aboveground competition for CO_2 and light. Rather than always using conservative strategies in low resource conditions, if nutrient patches are transient, it may be more efficient to build fine roots that are short-lived and low-cost so that they can proliferate quickly into a new resource patch, die once resources are depleted, and then shift that investment to other areas (Adams et al. 2013). If resource patches are big enough or have a long enough duration, though, then lifespans of newly produced fine roots in nutrient- and water-rich patches may be extended and fine roots may present with a more conservative strategy (Pregitzer et al. 1993). For example, in sugar maple sampled across a N mineralization gradient, fine root longevity was found to be longer and turnover lower where N mineralization rates were higher, even in relatively acquisitive fine roots with respect to root N and RSR (Burton et al. 2000). Thus, it is possible that populations of *Q. rubra* representing the more conservative side of the spectrum inhabited areas with lower and/or more constant resource availability. As this economics axis, which typically is demonstrated in studies across species, was still present within *Q. rubra*, it seems likely that this trade-off can be important across species ranges as well. Future work might investigate if similar trade-offs exist even within the root systems of individual trees.

The second axis, dominated by variation in branching intensity, explained 30.34 % of the variation in the data. In ectomycorrhizal species like *Q. rubra*, greater BI could reflect increased fungal colonization due both to increased surface area for symbionts and carbohydrate transfer through sieve tubes as well as exudation of ethylene and auxin by ectomycorrhizal fungi to induce root branching (Weigelt et al. 2021, Yan et al. 2022). This elevated number of root tips in trees allocating a higher proportion of C to symbionts rather than their own root growth for soil exploration could be why in the whole-core samples, spanning a broader range of BI, a negative relationship was found between SRL and BI. However, as SRL also has a relatively high loading on this axis (0.359; Table 1.4), higher BI could also be indicative of more root foraging with both long exploring roots and many root tips for quick resource uptake. In ectomycorrhizal symbioses, not dependent on higher cortex-to-stele ratio, it may be possible to have high BI and low SRL, favoring fungal uptake, high BI and high SRL, favoring soil exploration and fungal uptake, or low BI and high SRL, favoring soil exploration and root uptake.

Across all woody species, there is evidence that ectomycorrhizal species like *Q. rubra* will respond to nutrient hotspots with more fungal hyphal growth than fine root growth, which would affect the range of fine root strategies found here. Because the ectomycorrhizal symbiosis evolved more recently and repeatedly than arbuscular mycorrhizal associations, it may show greater foraging plasticity by fungal partners while arbuscular mycorrhizal foragers tend to show more fine root trait diversity (Schaffer-Morrison and Zak 2023). Reliance on fungal nutrient uptake is likely highest in ecosystems with predominantly organic nutrients and slow nutrient cycling (Ma et al. 2021), such as the cold temperate sites investigated near the northern limit of this study. On the other hand, when more inorganic N is available, it becomes efficient for trees to do the bulk of nutrient uptake themselves. This has been found in both ecto- and arbuscular mycorrhizal trees across many ecosystem types following N addition and in *Q. rubra* across preexisting soil inorganic N gradients (Ma et al. 2021, Pellitier et al. 2021b), and may explain why both root economics and potential fungal symbiosis trade-off axes, as well as high variation in BI, were found here despite the constrained variation offered by a single species. More investigation will be needed to fully understand how RD and BI reflect economics strategies, mycorrhizal investment, or other factors entirely in ectomycorrhizal species, as well as how differences in colonizing fungal community composition shape fine root strategies.

The third principal component, though less important (explaining 13.74 % of the variation; Table 1.4), describes a trade-off with thinner, longer roots at one end and roots with greater branching intensity and higher specific respiration rates at the other. Again, this could be related to differences in ectomycorrhizal reliance, with higher BI and RSR indicating greater fungal reliance and higher SRL indicating more use of fine roots. Locations on this axis may shift depending on depending on type or rate of resource availability, as discussed above. If BI in ectomycorrhizal species reflects mycorrhizal dependence, then again, this relationship with RSR could be explained by increased maintenance respiration associated with the chitin-rich cell walls in colonizing fungi (Trocha et al. 2010)**.** Individuals may either extend their belowground zone of influence by investing in their own fine root growth or more so in fungal hyphal networks, leading to higher branching and in a small portion of the data, increased RSR.

Thus, the eigenvectors resulting from our PCA could show two potential acquisitive strategies within *Q. rubra*, a “do it yourself” strategy characterized primarily by high fine root N and RSR and a mycorrhizal investment strategy shaped by many root tips (Fig. 1.6; Bergmann et al. 2020, Yan et al. 2022). This does not perfectly match with the fungal collaboration axis proposed by Bergmann et al. (2020), describing a trade-off between SRL and RD. Here, we have investigated an ectomycorrhizal species whereas most of their data sources investigated arbuscular mycorrhizal species and did not include BI. Instead, our findings are better described by the ectomycorrhizal fungal symbiosis dimension proposed by Yan et al. (2022), with variation in BI as the primary determinant of a sample’s location along the axis. This dimension may be tested further by incorporating measurements of mycorrhizal community composition and biomass as a proxy for C allocation to symbionts. Conversely, in areas of lower or more stable nutrient availability, fine roots may exhibit a more conservative strategy with higher tissue density. This hypothesized explanation for the results of our PCA is depicted in Figure 1.7.

1.4.4 Limitations

A few limitations in this study make it challenging to draw definitive conclusions about the root economics space in *Q. rubra*. One is that because we measured fine root respiration rates, we limited our samples to only the roots with the highest respiration, surface fine roots ≤ 1 mm in diameter. As such, the vast majority were between 0.3 and 0.4 mm in diameter. Sampling from such a narrow diameter range makes it difficult to detect relationships between diameter and the other traits. The economics space discussed in the conclusion can thus only be applied to the finest roots.

Secondly, because we conducted N and respiration measurements on only the hand-picked respiration-only samples, we could not analyze these in relation to the more comprehensive whole-core samples. This led to a discrepancy in the results, where positive relationships were revealed between SRL and BI in the respiration-only samples used for the correlation matrix and PCA (Fig. 1.4), while the whole-core dataset showed a negative correlation between these variables (Fig. 1.3). Fine roots picked by hand tended to be more branched with a higher proportion of samples above 1.0 tips cm^{-1}, which represents a small portion of the overall graph and could have resulted in the positive correlation found. Distinguishing whether the respiration-only samples or the whole-core samples were more representative of the actual BI is challenging, as branched roots may be more noticeable and picked out in the field, but roots leftover may be more broken from the excision process, such that they lose their branching structure. Thus, correlation matrix and PCA results regarding branching in particular should be interpreted with caution.

Lastly, although our PCA results match those from other studies finding that across species, mycorrhizal colonization may drive a second trade-off axis (Comas and Eissenstat 2009, Liese et al. 2017, Ma et al. 2018, Paradiso et al. 2019, McCormack and Iversen 2019, Bergmann et al. 2020, Han and Zhu 2021, Li et al. 2021, Yan et al. 2022), we can only speculate about whether this axis explains our results. Neither mycorrhizal colonization nor composition were assessed. These results do suggest that a thorough inclusion of mycorrhizal colonization and exploration type would be beneficial to further study of fine root trait strategies, as this is a strong candidate driver of variation in BI along the fungal symbiosis axis, and the unexpected negative relationships of RD with RTD and RSR.

1.4.5 Conclusion

This study investigated fine root traits and respiration in *Q. rubra* across the Midwest to better understand foraging strategies and their effects on the fine root respiration flux. We hypothesized that (H1), morphological trait trade-offs would be multidimensional, with one axis describing more conservative roots with high tissue density and the other describing a fungal collaboration trade-off with longer, thinner roots at one end and those with greater average diameter and branching intensity at the other. Our results did describe multi-dimensional trade-offs, but they were primarily between high specific root length on one end and high tissue density and branching intensity on the other, and secondarily between longer, thinner samples and those with higher average diameter with an emphasis on diameter variation. When incorporating root respiration and N, the most important trade-offs became first, longer, thinner samples with greater respiration and N concentration, versus those with denser fine roots and second, variation in the number of root tips per unit root length. Thus, our results aligned with H2, that as root specific respiration and N reflect work being done, roots with higher values would fall on the acquisitive end of the root economics axis and opposite to denser roots on the conservative end. The second axis, characterized by variations in branching intensity, may represent an ectomycorrhizal fungal symbiosis dimension. Although few studies have investigated these trade-offs within a single species, we observed very similar trade-offs in *Q. rubra* to those reported across ectomycorrhizal species, suggesting comparable controls on inter- and intraspecific trait variation. Lastly, root specific respiration appears to be driven largely by a trade-off between root N and tissue density, describing a difference in rate of return on investment. Shifts in economics strategies across environmental gradients may therefore influence fine root respiration, an important and poorly understood C flux out of terrestrial C sinks.

1.5 Tables and Figures

Table 1.1. List of site codes and location names for 14 sites across the Midwest gradient, along with site latitude and longitude coordinates, mean annual temperatures (MAT; °C), basal area (BA; m^2 ha^{-1}) and trees per hectare (TPH; trees ha^{-1}) obtained from 6 plots per site with no diameter limit cutoff. Relative dominance of *Quercus rubra* is calculated as a percentage of total basal area, and other species are those representing greater than 15 % of the total basal area, from highest basal area to lowest.

Site code	Location name	Lat	Long	MAT	BA	TPH	% *Q. rubra*	Other species*
NOWI	Nicolet NF	45.993	-89.073	3.81	31	7071	89	NA
DFKE	Dow Forest	47.426	-88.059	4.18	24.9	10645	23	ABBA, ACRU, ACSA, TSCA
OTUP	Ottowa NF	46.407	-89.218	4.26	14.2	3109	62	ACRU
BPUP	Baraga Plains	46.656	-88.590	4.36	23.4	2360	79	PIRE
HOKE	Tech Trails, Houghton	47.105	-88.531	5.07	30.6	3259	61	ACRU, ACSA
HWUP	Hiawatha NF	46.411	-86.813	5.11	15.3	1332	83	NA
NOLP	Atlanta SF	44.921	-84.123	6.29	21	3742	82	ACRU
MILP	Huron NF	44.257	-85.769	6.82	24.9	1812	69	ACRU
NEIA	Yellow River SF	43.172	-91.242	7.83	31	1895	59	ACSA
SOLP	Waterloo State Rec Area	42.332	-84.112	8.44	23.7	4224	68	ACRU
MIIL	Mackinaw River SFWA	40.562	-89.316	10.43	24.1	3775	44	NA
SOIN	Hoosier NF	39.008	-86.295	11.56	23.3	2675	36	QUAL
SOIL	Trail of Tears SF	37.493	-89.339	13.52	24.9	1626	46	QUAL
WETN	Montgomery Bell SP	36.091	-87.281	13.97	22.2	2179	14	QUAL, LITU, QUVE

*ABBA: *Abies balsamea*, ACRU: *Acer rubrum*, ACSA: *Acer saccharum*, LITU: *Lirodendron tulipifera*, PIRE: *Pinus resinosa*, QUAL: *Quercus alba*, QUVE: *Quercus velutina*, TSCA: *Tsuga canadensis*

Table 1.2. Summary of ANCOVA results for relationships between traits quantified from the respiration-only samples and specific respiration rate with campaign month as the categorical factor. Significant results are bolded (α = 0.05). RTD is root tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), BI is branching intensity (tips cm^{-1}), RD is average root diameter (mm), N is root nitrogen (mg g^{-1}), and RSR is root specific respiration (nmol CO_2 g^{-1} s^{-1}).

Independent	Source	df	*P*-value
RSR	ln(SRL)	1	**0.001**
	Month	1	0.172
	ln(SRL) x Month	1	0.309
RSR	BI	1	0.773
	Month	1	0.226
	BI x Month	1	0.821
RSR	N	1	**<0.001**
	Month	1	0.381
	N x Month	1	**0.024**
RSR	RTD	1	**<0.001**
	Month	1	0.444
	RTD x Month	1	0.173
RSR	RD	1	**0.035**
	Month	1	0.393
	RD x Month	1	0.835

Table 1.3. Principal components that cumulatively explain 99 % of the variation in the root morphological traits from principal component analysis on the whole-core samples. Each component is displayed with the percent variation it explains and variable loadings. Loadings greater than 0.4, an arbitrarily chosen cut-off, are bolded. RTD is root tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), BI is branching intensity (tips cm^{-1}), and RD is average root diameter (mm).

Source	PC1 (64.38 %)	PC2 (29.50 %)	PC3 (5.25 %)
ln(SRL)	**-0.538**	**0.437**	-0.251
BI	**0.571**	0.141	**-0.906**
RTD	**0.603**	0.042	**0.484**
RD	-0.146	**-0.887**	-0.229

Table 1.4. Principal components that cumulatively explain 81 % of the variation in the root traits from principal component analysis on the respiration-only samples. Each component is displayed with the percent variation it explains and variable loadings. Loadings greater than 0.4, an arbitrarily chosen cut-off, are bolded. RTD is root tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), BI is branching intensity (tips cm^{-1}), RD is average root diameter (mm), N is root nitrogen (mg g^{-1}), and RSR is root specific respiration (nmol CO_2 g^{-1} s^{-1}).

Source	PC1 (37.37 %)	PC2 (30.34 %)	PC3 (13.74 %)
RSR	**0.414**	-0.190	**-0.416**
ln(SRL)	**0.520**	0.359	**0.423**
BI	0.062	**0.535**	**-0.630**
N	**0.500**	-0.053	-0.344
RTD	**-0.550**	0.255	-0.282
RD	-0.037	-0.037	-0.232

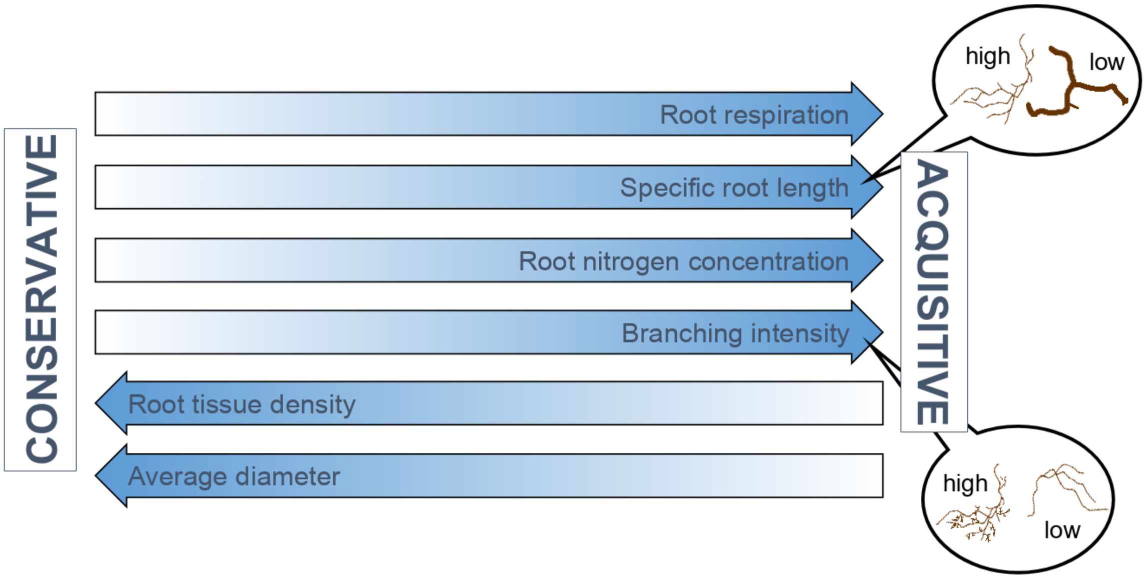

Figure 1.1. Conceptual diagram of the hypothesized root economics spectrum tested here. Darker shades of blue indicate higher trait values, and speech bubbles are used to further depict specific root length and branching intensity.

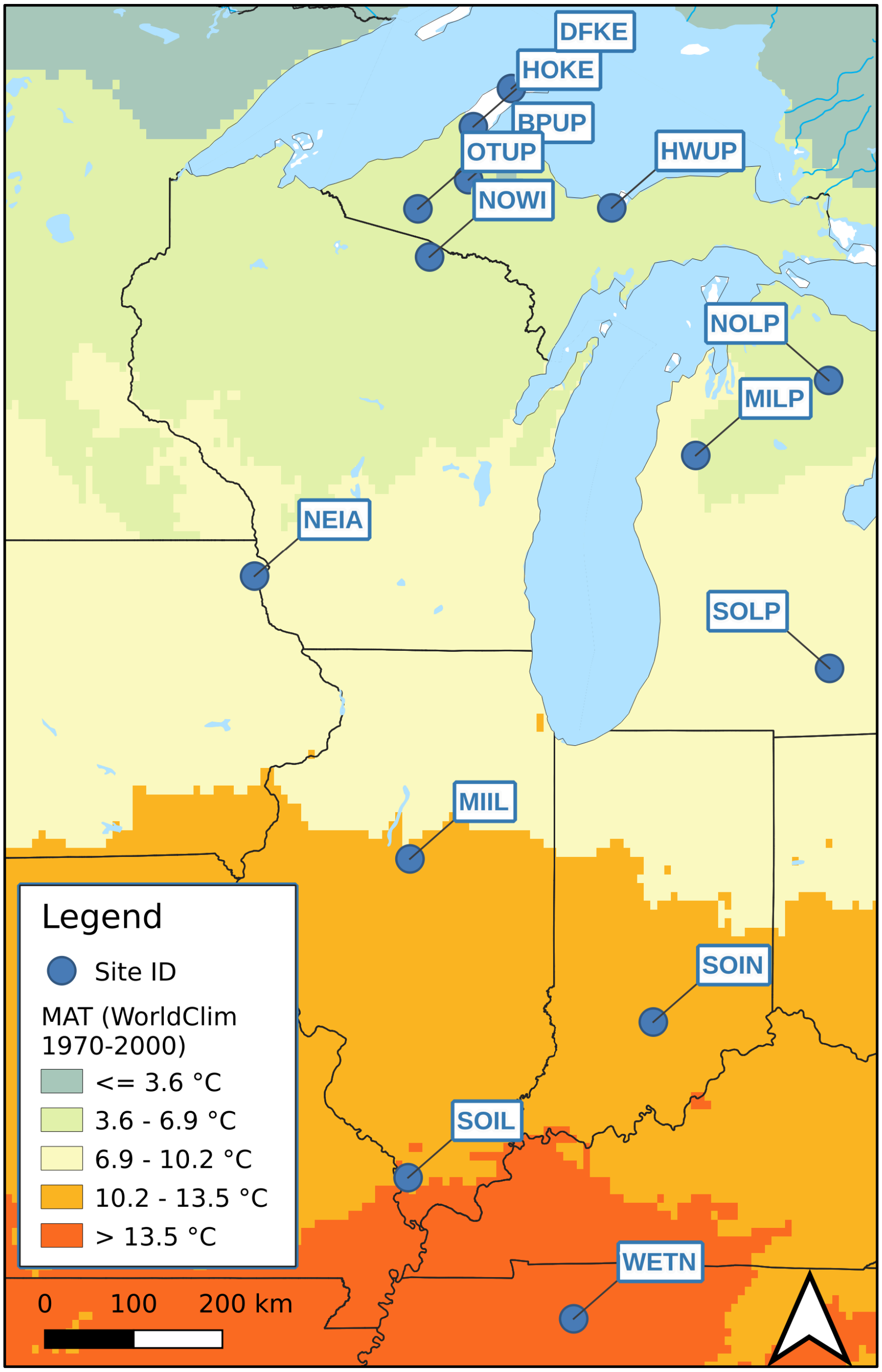

Figure 1.2. Map displaying the 14 study sites across the Midwest overlaid on mean MAT values from 1970 - 2000 (Fick and Hijmans 2017).

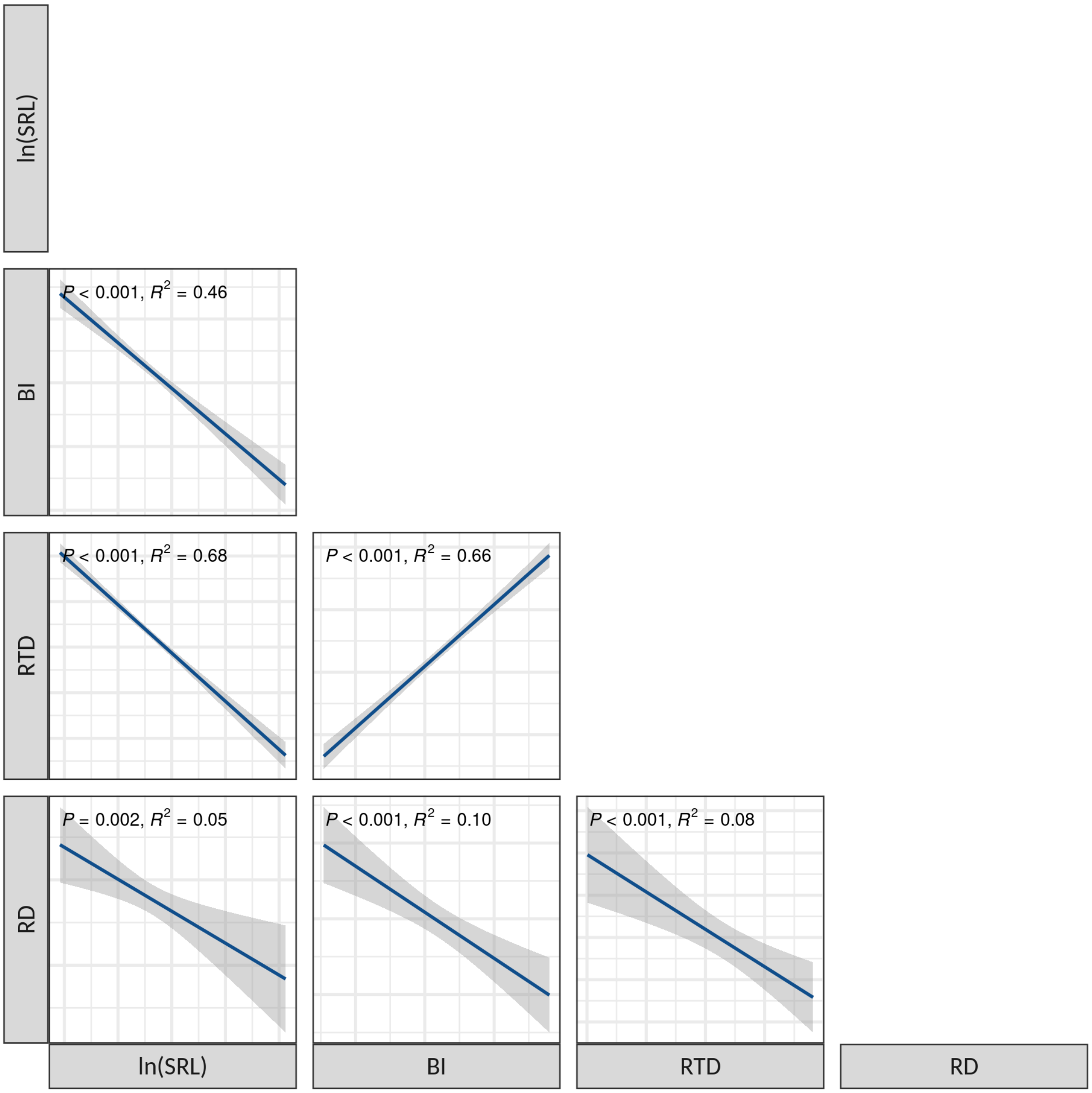

Figure 1.3. Correlations between all fine root morphological traits using the whole-core samples (n = 168). Gray shading is the standard error around the line and significance level is α = 0.05. RTD is root tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), BI is branching intensity (tips cm^{-1}), and RD is average root diameter (mm).

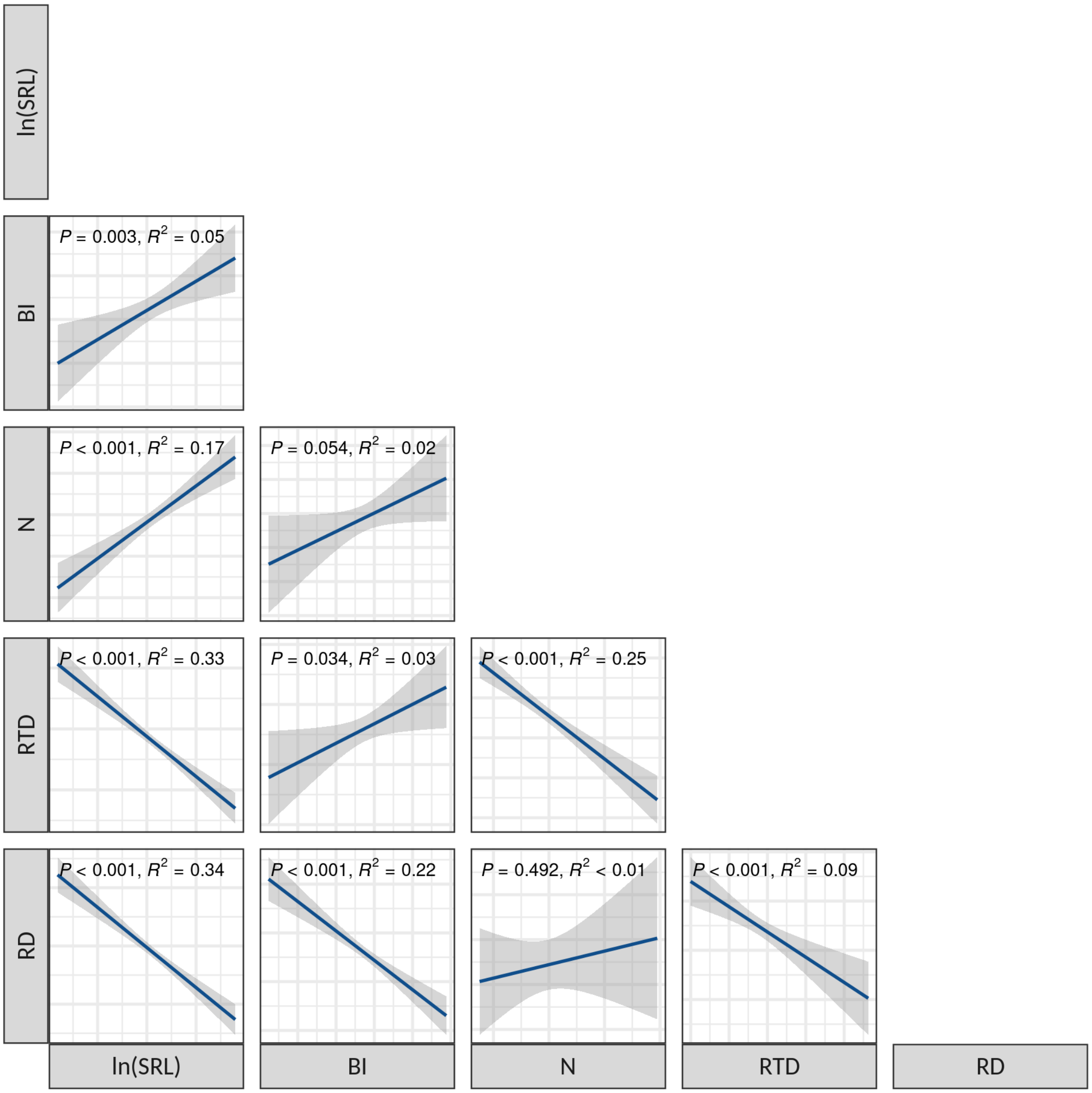

Figure 1.4. Relationships between fine root traits, including N, from the respiration-only samples (n = 168). Gray shading is the standard error around the line and significance level is α = 0.05. RTD is root tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), BI is branching intensity (tips cm^{-1}), RD is average root diameter (mm), and N is root nitrogen (mg g^{-1}).

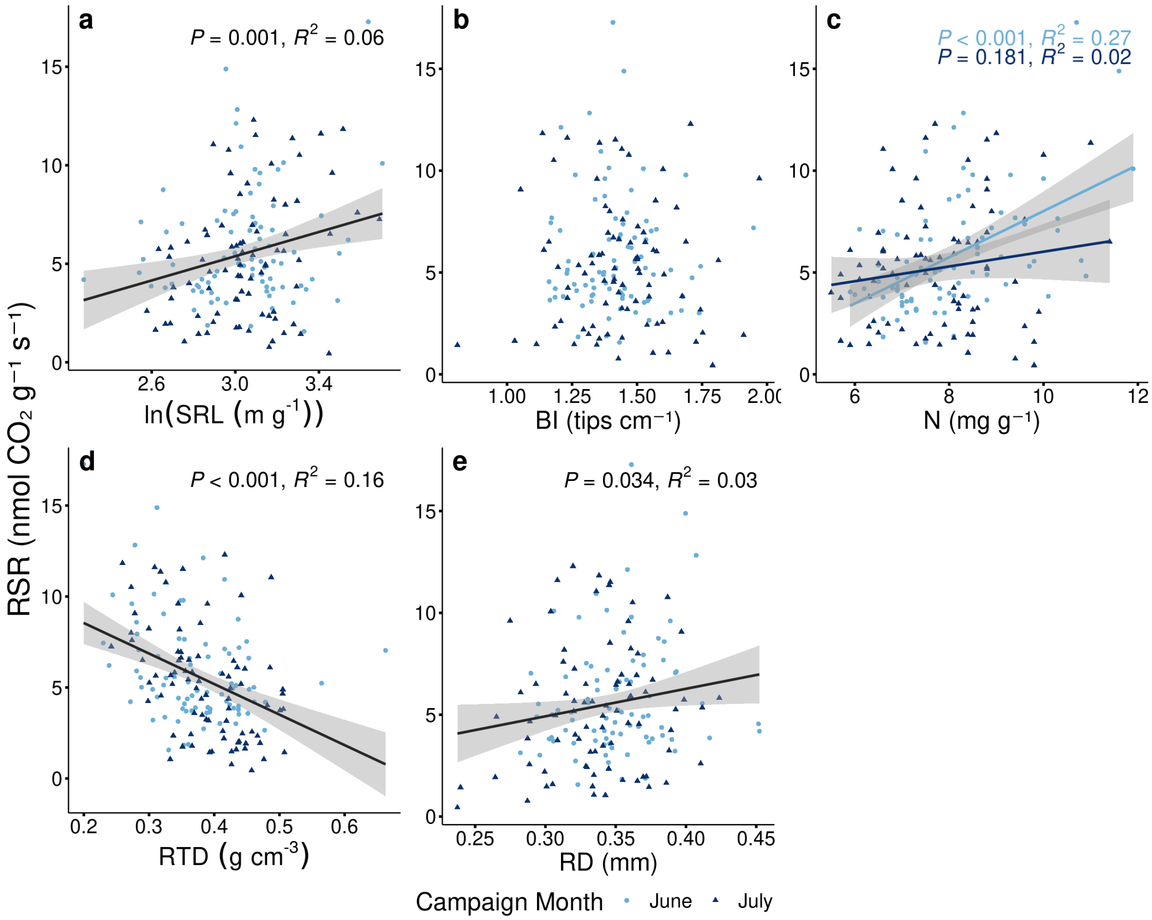

Figure 1.5. Relationships between specific fine root respiration rates at 18 °C and traits in June and July from the respiration-only samples (n = 168). Graphs with regression lines pictured show significant relationships (α = 0.05), and gray shading is the standard error around the line. Those depicted with two lines exhibited a significant interaction between the explanatory trait and campaign month. BI is branching intensity, RTD is root tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), BI is branching intensity (tips cm^{-1}), RD is average root diameter (mm), N is root nitrogen (mg g^{-1}), and RSR is root specific respiration (nmol CO_2 g^{-1} s^{-1}).

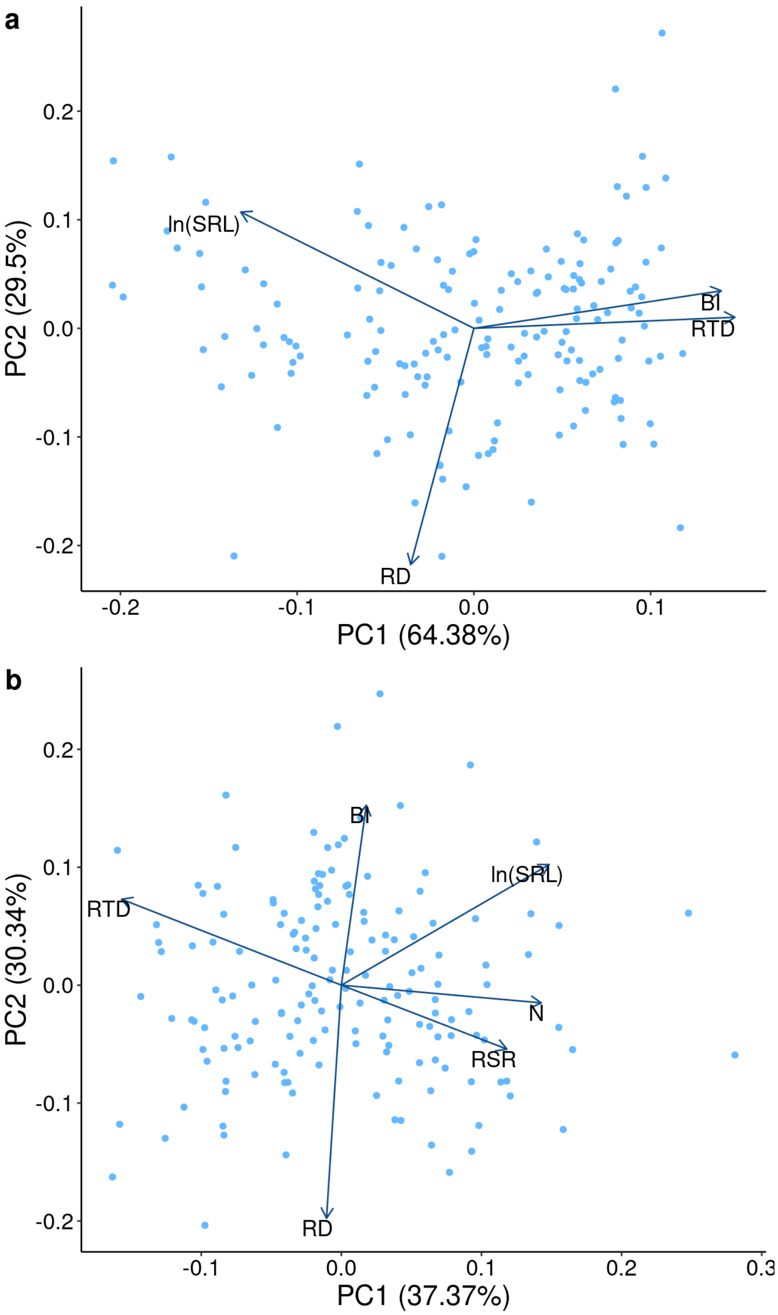

Figure .. Results from PCA on fine root morphological traits from the whole-core dataset, displaying eigenvectors from the two principal components explaining the most amount of variation in the data (a). PCA using the respiration-only dataset (b). Axes include the percentage of variation explained by each PC, RTD is root tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), BI is branching intensity (tips cm^{-1}), RD is average root diameter (mm), N is root nitrogen (mg g^{-1}), and RSR is root specific respiration (nmol CO_2 g^{-1} s^{-1}).

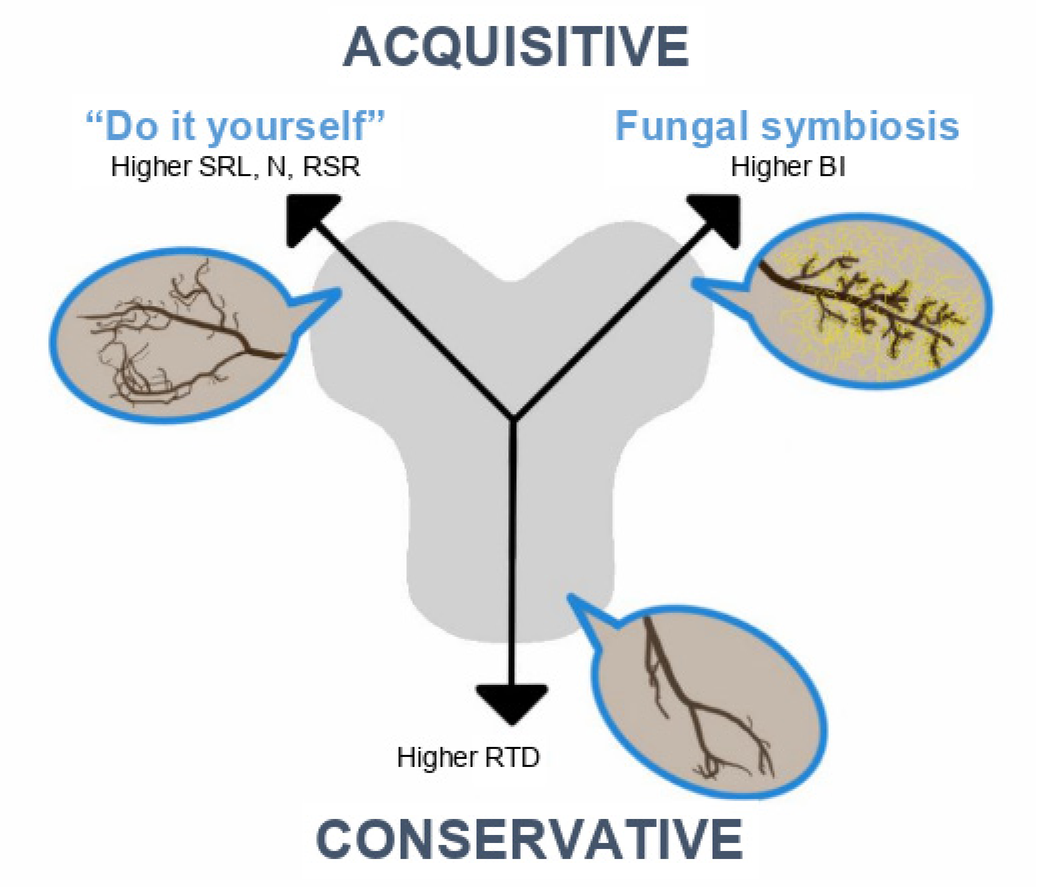

Figure 1.6. Conceptual diagram depicting the hypothesized explanation for the PCA results shown in Figure 1.6. The “do it yourself” and fungal symbiosis axes are both shown to be acquisitive strategies (Bergmann et al. 2020, Yan et al. 2022), and high RTD represents the conservative strategy. Acquisitive fine root systems could either be long and thin with high N and respiration, or have many root tips with a greater reliance on mycorrhizal hyphae for soil exploration. High RSR is most associated with the do it yourself strategy. Average diameter is left out as variation in this trait across our data set was low, and it was not an important contributor to any of the first three principal components (Table 1.4). RTD is root tissue density (g cm^{-3}), SRL is specific root length (m g^{-1}), BI is branching intensity (tips cm^{-1}), N is root nitrogen (mg g^{-1}), and RSR is root specific respiration (nmol CO_2 g^{-1} s^{-1}).

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