Intraspecific variation in leaf and root traits across nutrient and light gradients in coffee agroforestry systems

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science
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2016

Abstract

Functional traits can evaluate crop responses to farm-level management practices, however there are considerable uncertainties as to the extent and drivers of trait variation. In a coffee agroforestry system I examined three shade and four nutrient treatments, in order to i) quantify intraspecific trait variation of coffee leaves and roots along soil fertility and light gradients, and ii) test if the functional biology of coffee, as described by its position along a known functional trait spectra, is best predicted by soil nutrients or light. Low light led to high intraspecific variation within plant photosynthetic rates while high light systems resulted in reduced intraspecific variation across all leaf traits. High fertilization produced lower amounts of intraspecific root variation. Finally, functional traits correlated along a resource gradient as expected. Agroforestry systems then have the potential to increase within species variation, which has important long-term consequences for the structure and function of agroecosystems.
Acknowledgments

I am very thankful to my committee members Marney Isaac, Adam Martin and Tat Smith for their guidance and constant support throughout the planning, collection, analysis and writing process. Their constant reassurance helped to quiet my insecurities and helped me to produce a piece of work I can be proud of.

Thank you to my advisor and field site supervisor at CATIE, Karel van der Meerche for his support and guidance in the experimental design of my thesis. Special thanks to Alvaro for his patience and guidance in the field. My work would have been a much more trying experience if you were not there to lend a hand and be a friendly smile. As well I am grateful to Patricia Leandro for the generous use of lab space at CATIE.

I am very thankful to everyone who has helped over the past year and a half, including my colleagues Jessie Furze, Kira Borden, Adam Dickinson and Brent Coleman, who shared with me their insights, experience and guidance. Finally, I want to thank all my friends and family who have been there to support me, calm me and inspire me.
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Chapter 1
Introduction

1.1 Background

Agroforestry is an economically and environmentally sustainable agricultural practice that incorporates trees into cropped agricultural systems (Atangana et al., 2014). Research strongly suggests that the integration of trees into agricultural systems increases nutrient and water capture and cycling processes (Jose, 2009), which in turn reduces farmers’ reliance on fertilizers and irrigation (Khanna, 1998). Other studies have also shown that the integration of trees greatly increases on-farm carbon sequestration (Nair et al., 2009) while maintaining biodiversity through habitat provisioning (Bhagwat et al., 2008). In addition to evidence indicating that agroforestry confers these specific benefits, generally, agroforestry systems result in biologically complex agroecosystems that maintain a multitude of multi-trophic interactions, as well as heterogeneous soil and light environment (García-Palacios et al., 2013; Jager et al., 2015). Yet while the environmental effects of integrating trees into cropping systems have been relatively well documented (Tscharntke et al., 2011), there still remain considerable uncertainties regarding how different crops respond to the presence of trees.

To date, crop performance in agroecosystems has typically been evaluated by comparing yield or other physiological parameters in monoculture, with the same parameters in agroforestry arrangements (Campanha et al., 2004). While these studies have been critical in informing our understanding of the dynamics and sustainability of alternative agricultural management, novel approaches to evaluating ecological interactions in terrestrial ecosystems may provide additional insights into how crops respond to environmental variability. In particular, contemporary ecological literature and theory suggests that information on the functional traits of crops may provide novel insights into the causes and consequences of crop responses to environmental change or management (Diaz et al., 2004; Garnier & Navas, 2012; Martin & Isaac, 2015; Wood et al., 2015).

Functional traits are the morphological, phenological or physiological features of plants and plant organs, which mechanistically underpin plant responses and effects on the environment (Garnier et al., 2012; Pérez-Harguindeguy et al., 2013; Wood et al., 2015). In terrestrial ecology, considerable efforts have been made to identify the key dimensions of functional trait variation
in plants, with arguably two of the most important suites of traits being aboveground leaf traits (Wright et al., 2004), and belowground root traits (Martin & Isaac., 2015; Tobner et al., 2013). One of the most widely recognized trait relationships is the hypothesized “leaf economics spectrum” (LES) (Wright et al., 2004). The LES suggests that all plants fall along a spectrum of life-history strategies, ranging from fast-growing, short-lived “resource acquiring” species on one end, to slow-growing, long-lived “resource conserving” species on the other (Wright et al., 2004). At the “resource acquiring” end of the LES, plants express high leaf nitrogen (N) concentrations, high rates of leaf-level photosynthesis and respiration, short leaf lifespans, and low specific leaf area, while species at the opposite end of the LES are characterized by the opposite suite of traits (Wright et al., 2004). Research has shown that a species’ position along the LES is dependent on multiple environmental factors including climate (Nicotra et al., 2010), soil fertility (Jager et al., 2015; Ordoñez et al., 2009) and local light availability (Gagliardi et al., 2015; Matos et al., 2009).

To date, our understanding of how and why LES traits influence the functional biology of plants has been based on species comparative studies that evaluate i) patterns of interspecific trait variation (Wright et al., 2004), ii) correlations between species’ traits and realized plant performance (i.e. growth, mortality, and reproduction rates) (Reich, 2014), and iii) relationships between species’ traits and rates of ecosystem functioning (García-Palacios et al., 2013; Wood et al., 2015). These studies have largely focused on quantifying these patterns with respect to interspecific trait variation (Poorter et al., 2012; Reich, 2014; Wright et al., 2004). However, in agroecosystems intraspecific trait variation has also been shown to reflect the functional biology of individuals within a crop species (Bolnick et al., 2011); differences which in turn are dictated by local environmental conditions and are related to realized plant performance such as crop yield (Franck & Vaast, 2009; Gagliardi et al., 2015). In agroecosystems generally then, there is reason to expect that information on intraspecific variation in functional traits, and LES traits in particular, might inform our understanding of how crops respond to management. This is especially true in agroforestry systems where light and fertility gradients (key drivers of LES trait variation) are expected to be particularly heterogeneous at fine spatial scales.

To date, the majority of trait-based studies, particularly the few that exist from agroecosystems, have focused on leaf traits (Gagliardi et al., 2015), but belowground traits are progressively receiving more attention as ecologically important determinants of plant functioning (Bardgett et
al., 2014; Tobner et al., 2013). Certain root traits have been found to be highly correlated with rates of nutrient and water uptake, suggesting they are vital indicators of plant functioning under different environmental conditions (Tobner et al., 2013). In particular, recent studies hypothesize that, near-analogous to the LES, high specific root length (SRL) and root N concentrations are indicative of resource acquiring life-history strategies, while the opposite traits should be expressed by resource-conserving plants or species (Bardgett et al., 2014; Prieto et al., 2015).

One economically and environmentally important crop is coffee (Blackman & Naranjo, 2012) which I will use as my model system. In 2013, Central America produced 962,044 tonnes of green coffee, with Costa Rica contributing 76,819 tonnes (FAO, 2013). While coffee is no longer the primary export crop of Costa Rica (FAO, 2015), it remains one of the top agricultural commodities in the country (Blackman et al., 2012). Based on its distribution in natural ecosystems, coffee is generally regarded as a shade-tolerant species (DaMatta, 2004); from a management perspective then, it was traditionally grown in agroforestry system as “shade-coffee” (Beer et al., 1998). Coffee in Costa Rica was grown this way until roughly the 1980s when increased market demand caused a shift in management practises towards high yielding “technified” monoculture coffee. This entailed management regimes with minimal incorporation of shade-trees and intensive applications of fertilizers and other agrochemicals (Blackman et al., 2012). However, considerable empirical evidence has since shown that this form of production is directly linked to a number of negative environmental impacts, including loss of biodiversity (Bhagwat et al., 2008) and human health risks (Ma et al., 2014). In sum, these findings have more recently catalyzed a shift by many farmers back to agroforestry practises (Blackman et al., 2012).

1.2 Research question/hypotheses

How does variation in coffee leaf and root traits change along a soil fertility and light gradient in agroforestry systems?

To evaluate this question, I conducted a field study on a small-scale coffee farm in Costa Rica, where shade-tree and nutrient management regimes results in 12 different growing conditions across three light regimes (monoculture, pruned *Erythrina* shade tree and unpruned *Erythrina* shade tree), and four soil fertility levels (50, 110, 170 and 230 kg N/ha). I hypothesize that:
1) Coffee leaf traits will covary following the same patterns as those proposed by the LES.
2) Coffee leaf traits will vary systematically across shade treatments, while root functional traits will vary systematically across nutrient treatments.
3) Variation will be greater for leaf traits under shade trees, as there is more partitioning of light under a tree creating heterogeneous conditions
4) Variation will be greater for root traits under low N additions with shade trees, since these conditions promote roots to forage more for soil nutrients (in comparison to monoculture, high nutrient treatments).

1.3 Significance

From a basic science perspective, evaluating intraspecific leaf and root trait covariation across nutrient and light gradients will be critical in developing a comprehensive understanding of the causes and consequences of trait variation within plants (Albert et al., 2010; Bolnick et al., 2011; Gagliardi et al., 2015). While considerable evidence exists to support the existence of a generalized LES (Ordoñez et al., 2009; Wright et al., 2004), research on belowground functional traits has lagged in this regard. Data from my research will be critical in testing the hypothesis that root traits covary across a similar spectrum (Bardgett et al., 2014). From an applied perspective, testing my hypotheses will make two key contributions to the agroecology and applied ecology literature. First, one of the prospective limitations of shade-coffee is reduced coffee yields as compared to technified monocultures (Campanha et al., 2004). Testing hypotheses using a functional trait-based approach will provide important management-related insights into how and why management influences the ability of coffee to reproduce, grow and adapt to heterogeneous environments (Wood et al., 2015). Secondly, my research will represent among the first applications of the theories and principles of trait-based ecology into questions in agroecology. I anticipate that this novel research approach and the results of my research will further support the benefits of trait-based approaches for understanding the structure and function of agroecosystems globally (Martin & Isaac., 2015).
Chapter 2
Literature Review

2.1 History of coffee

Coffee (Coffea arabica L.), has traditionally been grown under tree canopies with moderate to high shade (Jha et al., 2014), and is known as shade-grown coffee. Shade provides coffee plants with a stable microclimate which in turn regulates water evaporation and leaf temperatures (Siles et al., 2009), as well as provides habitat for many bird species that help to control pest populations (Ponte, 2002). Shade trees grown alongside coffee also provides soil with nutrients essential for plant growth such as nitrogen (N) and phosphorus (P) (Richards & Méndez, 2014), through the increased litter fall of leaves and woody plant materials. In the 1970s, coffee production shifted to more intensified management styles to increase yields in order to meet the increasing worldwide demand for coffee (Beer et al., 1998) with global peak production occurring in 1992 (FAO, 2015). These intensified management styles required higher amounts of light, which entailed coffee monoculture management systems with few or no shade trees (Jha et al., 2014). This form of management became preferred as it resulted in higher yields as compared to traditional shade-tree management systems (Beer et al., 1998; Campanha et al., 2004). However, these higher yields also demanded the application of N-based fertilizers and agrochemicals, in order to compensate for soil nutrient exhaustion and pest infestation (Campanha et al., 2004; Jha et al., 2014).

Clear cutting of trees for agriculture and livestock production has been linked to environmental concerns such as lowered carbon sequestration (Jose, 2009), increased soil erosion and subsequent water pollution (Ivanilda de Aguiar et al., 2010), and loss of biodiversity from tropical forests (Bhagwat et al., 2008). Because of this, there has been a rise in interest for environmentally conscious coffee production (Ponte, 2002) such as shade-coffee. While a recent awareness about environmental degradation and its long-term effects has seen a change in the global coffee market, with a greater desire for shade-grown coffee by consumers (Ponte, 2002), monoculture coffee production still accounts for 90% of all production in Costa Rica (Blackman et al., 2012).
One reason for the continued large scale production of monoculture coffee is the significantly higher yields it produces in comparison to shade-grown coffee (Campanha et al., 2004; Haggar et al., 2011; Soto-Pinto et al., 2000). For example, one study examined the yield from coffee plants in monoculture and shade systems which received the same amount of fertilization. Monoculture coffee outcompeted shade-coffee yield producing 2443 kg ha\(^{-1}\) of coffee compared to 515 kg ha\(^{-1}\), respectively (Campanha et al., 2004). Lower yields of shade grown coffee is a result of increased shade (i.e. less available photosynthetically active radiation (Charbonnier et al., 2013) and competition for soil resources (Casper & Jackson, 1997; Moreno Marcos et al., 2007) provided by the introduction of another species, such as a tree (Beer et al., 1998).

While there are many studies indicating that monoculture coffee produces higher yield than shade-coffee, these results are inconsistent and depend greatly on site conditions and management (Beer et al., 1998). Moreno Marcos et al. (2007) demonstrated that proper spatial and temporal intercropping arrangements of trees and crops can reduce light and nutrient competition. For example, optimal photosynthetic rates of C3 plants in this study occurred at 25-50% shade. Soil nutrients (total N and available P) also increased in the top layer of a soil (20 cm) under the canopy of a tree owing to the increased amounts of litter fall (Moreno Marcos et al., 2007). When these conditions are accounted for, shade-coffee can match and even outperform monoculture coffee (Haggar et al., 2011).

2.2 Coffee and agroforestry

One economically and environmentally sustainable alternative to monoculture coffee production is agroforestry (Atangana et al., 2014), where trees or other woody perennials are planted with crops (i.e. shade-coffee), in spatial or temporal arrangements (Nair, 1993). Specifically, the integration of a N\(_2\)-fixing shade tree grown alongside coffee plants can provide some of the soil N needed for plant growth (Khanna, 1998; Kurppa et al., 2010), as well as increased nutrients from natural litter fall and decomposition (Richards et al., 2010). Agroforestry can also increase photosynthetic capacity in plants (Franck et al., 2009) by reducing photoinhibition which is commonly seen in full sun coffee plants (Nunes et al., 1993). The use of shade trees also contributes to atmospheric carbon (C) sequestration, which would otherwise not
be seen in a monoculture system (Jose, 2009; Nair et al., 2009). Finally, trees can provide increased soil stability (i.e., reduced soil erosion) through intricate root systems (Ma et al., 2014). The integration of shade trees are also a key component in qualifying for eco-certifications schemes which would allow a farmer to receive payments for eco-system services as well as facilitate price premiums on certified coffee (Blackman et al., 2012). Agroforestry can also provide a certain amount of economic security to a farmer as there is less reliance on a single crop and more income from the fruit and timber of the trees which they are grown with (Beer et al., 1998). This may help farmers in years when the global market price for coffee is low or if yields are lower than expected.

The primary disadvantage of shade grown coffee in comparison to monoculture coffee is lower average yield. The reason for this lower yield can include an over-shading effect from the tree species if not properly pruned (Charbonnier et al., 2013), and competition for soil resources (Powell, 2003). Knowing how to properly prune and add nutrients is integral in increasing yield for shade-coffee (Campanha et al., 2004; Jha et al., 2014). One particular study demonstrated that proper management and selection of shade tree can reduce the yield gap between monoculture and agroforestry production (Haggar et al., 2011). Costa Rican coffee grown as a monoculture and under two different shade trees showed differences in their total productivity over a five year study. When fertilization was reduced in both monoculture and agroforestry systems, coffee grown under the cover of *Erythrina poeppigiana* showed greater yields in comparison to monoculture production (Haggar et al., 2011). While yield differences has been the focus of many studies, Vaast et al. found that while shade decreased productivity by 18%, it positively affected the size and composition of the bean, resulting in overall higher beverage quality (2006). This change in beverage quality could have important implications in the pricing of the coffee and the number of available coffee buyers especially with the emergence of a quality driven market. Therefore, when calculating the total economic output from shade and monoculture coffee, yield alone is not the best predictor as this is only a short-term economic gain.
2.3 Functional traits

Many studies have examined the consequences of integrating trees into agricultural systems (Tscharntke et al., 2011) with most studies focusing on yield and biomass as indicators of crop performance (Haggar et al., 2011). More contemporary research has indicated a functional trait approach can provide insight into evaluating ecological interactions among and within plant species (Martin & Isaac, 2015; Wood et al., 2015). In contrast to natural plant communities, where light and nutrient conditions cannot be controlled for, agroecosystems and associated management strategies directly manipulate soil and light environments, as well as plant diversity. These variable environments are expected to have considerable effects on functional trait expression and variation (Ordoñez et al., 2009), as plants adapt to changes in nutrient, water and light availability.

The introduction of trees into agricultural systems creates spatially and temporally heterogeneous light and soil environments (Charbonnier et al., 2013; García-Palacios et al., 2013; Hodge, 2004). A plant has the ability to adapt its morphological and physiological traits (phenotype) in order to suit the demands of a particular environment (Bradshaw, 2006; Wood et al., 2015). These trait combinations represent different ecological strategies which can be used to either acquire or conserve resources (Donovan et al., 2011). A single plant can therefore display a wide variety of functional traits, in order to suit a heterogeneous environment. Four key areas of trait variability have been identified and used in previous studies as response variables to environmental change and management techniques; i) maximum plant size metrics, ii) leaf traits iii) root traits and iv) reproductive traits (Martin & Isaac, 2015). For this study, leaf and root traits will be used exclusively as these have proved to be two of the most important, easily measureable and informative suites of traits.

2.3.1 Leaf traits

The leaf economics spectrum (LES) is the most widely recognized hypothesis that describes how leaf traits covary within one another, and how this variation correlates with interspecific differences in plant functional biology (Wright et al., 2004). In general, the LES suggests that all plant species exist along a continuum of fast-growing, short-lived “resource
acquiring” species on one end, to slow-growing, long-lived “resource conserving” species on the other (Wright et al., 2004). Leaf traits can provide information on a species’ position on this continuum. The LES is comprised of six leaf functional traits that covary with one another, including: 1) leaf mass per area (LMA); 2) light-saturated photosynthetic rates (A); 3) leaf N concentrations; 4) leaf phosphorus (P) concentrations; 5) leaf dark respiration (R); and 6) leaf lifespan (LL) (Wright et al., 2004). At the resource acquiring end of the LES, plants generally exhibit high A, R, leaf N, low LMA, and short LL, while species at the other end of the LES are characterized by resource conserving traits including as low A, R, and leaf N, high LMA, and long LL (Wright et al., 2004). These traits have been also correlated with a number of other functional leaf traits including leaf thickness and leaf dry matter content (LDMC) (Matos et al., 2009; Pompelli et al., 2012). Plants exhibiting resource acquiring traits could be seen as being more productive, especially in agricultural systems, as they grow faster and have a quicker return on investment (Reich, 2014). However, it is important to ensure that a plant will have a good return on investment. For example, investing a great amount of energy into root production for nutrient foraging is only beneficial if there is sufficient soil nutrients to then uptake. Therefore, resource acquiring traits are generally seen as beneficial in high resource environments (Reich, 2014).

2.3.2 Root traits

Fine roots (<2mm) play an important role in the uptake of soil water and nutrients (Tobner et al., 2013) and are highly plastic in response to resource supply (Eissenstat et al., 2000). Specific root length (SRL), fine root diameter (D), and branching intensity (BI) are all root traits which have direct influence on nutrient and water uptake (Tobner et al., 2013). Strong correlations have been found between these three root traits, whereby SRL and BI have been found to be positively correlated while SRL and D were negatively correlated (Comas & Eissenstat, 2009; Tobner et al., 2013). When we consider root traits as part of an economics spectrum similar to the LES, resource acquiring plants or species are hypothesised to exhibit high SRL, low root tissue density, high root N uptake and N content and vice versa for resource conservation (Bardgett et al., 2014; Mommer & Weemstra, 2012; Ostonen et al., 2007).
2.3.3 Environmental impact on trait expression

Environmental conditions (light, water availability and soil nutrients) have a direct impact on the expression of plant functional traits (García-Palacios et al., 2013; Jager et al., 2015; Ordoñez et al., 2009; Schöb et al., 2013). A coffee leaf’s position along the LES (from resource acquisition to resource conserving traits) is an indicator ecological strategy (Wright et al., 2004). In particular, light level has been found to have a significant effect on the expression of leaf traits (Gagliardi et al., 2015). In agroforestry systems, where there is over 30% shade, coffee plants tend to have larger, thinner leaves as compared to a full-sun grown coffee plant (Campanha et al., 2004; Pompelli et al., 2012). This is an example of how most leaf traits respond to light, in a classic sun versus shade leaf dichotomy (Givnish, 1988). However, as coffee is a shade-tolerant species, is it able to maintain high photosynthetic rates under moderate-high shade. High photosynthetic rates were reported in coffee plants which received up to 55% shade (Beer et al., 1998; Franck et al., 2009). Franck et al. (2009) demonstrated that coffee plants grown in shade up to 55% were able to maintain photosynthetic performance by investing more energy into the growth of leaf area (LA), for improved light capture. Under this scenario, coffee leaf traits are more likely to be found towards the resource acquiring end of the LES.

A leafs’ position along the LES is also closely related to soil fertility as demonstrated by Ordonez et al., (2009) who found that specific leaf area (SLA), leaf nitrogen (LNC) and leaf phosphorus concentration (LPC) all increased with increasing soil fertility (soil N and P). This study exemplifies the trade-off between resource acquiring and resource conserving leaf traits in relation to soil fertility, with high soil fertility leading plants to exhibit resource acquiring traits.

Root trait expression is also closely related to soil conditions, particularly soil moisture and nutrients (Ostonen et al., 2007). Mora and Beer (2013) demonstrated this phenomenon of spatial dispersion of roots through geostatistical modeling. Higher proportions of root length density (RLD, cm cm\(^{-3}\)) was found in areas of soil which also exhibited high nutrient content, indicating a unique nutrient acquisition strategy by coffee roots. SRL has also been shown to decrease significantly with fertilization, and responds negatively to decreases in light availability (Ostonen et al., 2007). In a study of wheat (Triticum aestivum L.) and jujube (Ziziphus jujube Mill.) where fertilization was kept constant for both species, intercropped plants which had
higher levels of shade were shown to have higher SRL and smaller D than plants which were grown alone (Wang et al., 2014). However, correlations among root traits and environmental gradient remains unclear, as root traits seem to vary with fertilizer type, and root diameter class (Ostonen et al., 2007; Tobner et al., 2013).

Since coffee roots can be extremely variable in their spatial distributions within a heterogeneous soil environment, this proposes a unique challenge in forming a sampling design. This becomes especially important to consider in agroecosystems, where there are multiple species vying for soil resources. In particular, the intercropping of multiple species causes increased belowground competition for nutrient resources (Zhang et al., 2013) which has been shown to decrease crop productivity (S Jose et al., 2000; Zhang et al., 2013). However, not all intercropped species show competitive interactions. Many studies performed in managed agroforestry systems, where tree-crop pairs have been selected for, showed enhanced crop performance (S Jose et al., 2000; Zhang et al., 2013) and resilience to environmental conditions such as drought (Schwendenmann et al., 2010).

2.3.4 *Intraspecific trait variation in crops*

Although the majority of trait-based research has evaluated patterns of trait variation across species, recent evidence also suggests these same patterns apply within species. This becomes particularly important in agricultural systems, where importance is placed on the productivity of a single crop. Similar trends were observed within-species leaf level traits (Gagliardi et al., 2015), with intraspecific trait variability being widest for physiological traits (A, R). As these traits are highly dependent on available light, intraspecific trait variation is also dictated by the same environmental variables that govern interspecific patterns of trait expression (Gagliardi et al., 2015). For example, one study of intraspecific leaf trait variation found that light level significantly influenced a leaf's position along the LES (Gagliardi et al., 2015), with high light transmittance being associated with resource conserving traits (reduced leaf size, mass-based photosynthetic rate and leaf nitrogen concentration). Intraspecific variation in root traits has not been well documented. One study which looked at intraspecific root variation in temperate tree species found that ontogenetic stage was an important factor, with juvenile plants showing higher SRL than adults (Tobner et al., 2013).
Variation of traits within a species depends largely on environmental conditions (Albert et al., 2010), and therefore management of agroecosystems play a large role in determining how a plant will respond. Management conditions which promote high levels of intraspecific trait variation have the potential for higher levels of resource partitioning, which would increase overall production (Wood et al., 2015). As well, environments which have abundant resources and management styles which promote resource acquiring traits will see higher overall plant productivity (Reich, 2014).

2.4 Gaps in the literature

Functional trait research has focused around natural plant communities and only recently have we seen an emergence of work focusing exclusively on managed systems (Gagliardi et al., 2015; Garnier et al., 2012). Understanding the expression of functional traits, from resource acquiring to resource conserving traits, over environmental gradients is paramount when deciding on appropriate management techniques in heterogeneous agroforestry systems. Root traits have begun to be examined in terms of falling onto an economic scale similar to the LES however results from these studies have been mixed (Bardgett et al., 2014; Mommer et al., 2012). As coffee is one of the worlds’ most produced and economically significant crops (FAO, 2015), information regarding intraspecific trait variation is critical for assessing management strategies as well as providing insight into how plants will respond to changes in management and environmental conditions (Wood et al., 2015).
Chapter 3
Site Description and Methodology

3.1 Site description

Our experiment was conducted at a 3-year old experimental agroforestry site, located on a large Rainforest Alliance certified coffee plantation located in Aquaires, Costa Rica (9° 56’ 19” N, 83° 43’ 46” W). The farm is situated at 1020 m above sea level on the slopes of Turrialba Volcano, where the dominant soil type are Andisols (USDA, 1999). Based on data from 1973-2009, mean annual rainfall at the site is approximately 3014 mm, with the lowest monthly rainfall (i.e. less than 200 mm per month) occurring between February and April (Taugourdeau et al., 2014). At the site, individual coffee plants are grown alongside both free-growing and pruned *Erythrina poeppigiana* (Fabaceae) trees, which are the most widely planted shade tree species used in agroforestry systems throughout Costa Rica (Haggar et al., 2011; Taugourdeau et al., 2014). At the farm, coffee is generally planted at densities of 5000 trees ha$^{-1}$, and intercropped with *E. poeppigiana* which are planted at densities of 3 trees ha$^{-1}$.

3.2 Experimental design and sample plant selection.

The experimental site consists of a total of four ha of land, which is then further divided into 12 experimental plots that are approximately 0.3 ha in size, and differ from one another in both shade management and N-based fertilization conditions. Specifically, all plots have been allocated one of three shade treatments nested within four fertility treatments (Fig. 1). The shade treatments consisted of 1) full sun (control) where no *E. poeppigiana* trees are present; 2) managed shade, where *E. poeppigiana* is fully pruned 2 times per year; and 3) full shade, where *E. poeppigiana* is free growing; the average height of the pruned *Erythrina* at the time of sampling was 2.38 m while average free growing *Erythrina* was 32.7 m. The nutrient treatments consist of 0, 110, 170 and 230 kg N ha$^{-1}$ yr$^{-1}$ applied as a complete fertilizer formula consisting of 24.5-0-15-5-0.43 (N-P-K$_2$O-Mg-B).

All three shade levels were nested within each of the four nutrient treatments for a total of 12 treatment combinations, which were then each replicated times times for a total of 36 study plots. Within each plot, a total of three reproductive coffee trees (i.e. those with visible cherries) were
Figure 1. Acquires Coffee Estates site layout, with nested shade (Full sun, Pruned *Erythrina*, Free growth *Erythrina*) within each fertilization (50, 110, 170, 230 Kg N ha\(^{-1}\) yr\(^{-1}\)) treatment.
chosen within 1-4 m of the closest shade tree. In the full sun plots, reproductive plants were chosen near the middle of the plot, to avoid interactions with neighboring treatments. In order to minimize potential trait variation owing to ontogenetic variation in coffee plant biology (Lavorel & Garnier, 2002), all coffee plants selected for this study were from three year old branches, as determined by experienced farm managers. Three year old plants were also chosen as these would be the next to be harvested and were known to be the most productive for cherry production. In total, we selected 105 individual coffee plants for functional trait analysis.

3.3 Environmental sampling

3.3.1 Soil analysis

All soil samples \((n = 105)\) were collected during the same day (per block) to reduce any differences in daily rainfall with a 0.5 L soil auger. Soil cores were taken 20 cm away from the base of each coffee tree to a depth of 20 cm, corresponding to the area where most fine roots occur (Mora & Beer, 2013). On the same day as collection, a 2 mm sieve was used to remove rocks and roots, and roots were set aside for further trait analysis. To measure gravimetric water content, a subsample of approximately 10 g from each soil core was weighed (wet soil mass) and then dried for a minimum of 24 hours at 105°C. The final dry weight of soil (dry soil mass) was recorded immediately after drying and used along with wet soil mass to calculate soil moisture \(\text{SM} = \frac{(\text{wet soil mass} - \text{dry soil mass})}{\text{dry soil mass}} \times 100\).

After soil moisture was calculated, the remaining soil was divided into two subsamples, one of which was kept frozen at -20°C and the other being air-dried to constant mass. Samples were then transported to the University of Toronto Scarborough for chemical analysis. First, 2 g of the air-dried sample was weighed, placed in an Erlenmeyer flask and extracted with 20 ml of Bray 1 to measure available soil phosphorus \((P, \text{mg kg}^{-1})\). A small amount of charcoal was added to each sample (as initial filtration showed signs of small particulates) and shaken for 5 minutes on an automated shaker table. Samples were then filtered into glass vials using P5 filter paper. For available inorganic nitrogen \((\text{NO}_x \text{ and } \text{NH}_4, \text{mg kg}^{-1})\), 2 g of the frozen soil was thawed and extracted with 20 ml potassium chloride \((2M \text{ KCl})\) and shaken for 30 minutes on an automated shaker table. Samples were filtered using Q2 filter paper. All filtered sample (for inorganic soil P and N) were then run through a Lechat QuikChem 8500 Series 2 Flow Injection Analyser.
(Lachat Instruments, Loveland, CO, USA). The final result provided measurements of available inorganic nitrogen in forms of nitrate (NO$_3$-N) and ammonium (NH$_4$-N) as well as inorganic P levels for each soil sample.

We then determined total soil N (mg g$^{-1}$) and C (mg g$^{-1}$) concentrations on a mass basis from the air dried samples. Air dried soil samples were ground using a Retsch ball mill (Retsch, Düsseldorf, Germany) and re-dried at 60°C for one hour prior to analysis on the EA. Elemental analysis was then performed on 55-65 mg of dried and ground soil using a ThermoFlash 2000 elemental analyzer (Thermo Scientific, MA, USA) which was calibrated using an aspartic acid standard which was run every 10 samples to ensure accuracy.

There were significant differences in inorganic soil N ($p<0.001$) and P ($p=0.001$) among the fertilization treatments with the exception of the 110 and 170 kg N ha$^{-1}$yr$^{-1}$ treatments, which had similar mean values (Table 1). There were also significant differences in total soil N ($p<0.001$) and C ($p<0.001$) among the fertilization treatments (Table 1). However, these differences were between the 230 kg N ha$^{-1}$yr$^{-1}$ treatment and the 50, 110 and 170 kg N ha$^{-1}$yr$^{-1}$ treatments only, where the 230 kg N ha$^{-1}$yr$^{-1}$ actually had lower total soil N and C. Since this study was interested in plant responses to environmental stimuli, inorganic soil N and P were used to verify our categorical fertilization treatment designations (50, 110, 170, 230 kg N ha$^{-1}$yr$^{-1}$).

3.3.2 Shade level

Digital hemispherical photographs were used to estimate incident radiation for each coffee plant. All photos were taken with a Nikon Coolpix 995 affixed with an FC-E8 fish-eye converter (Nikon, Tokyo, Japan) under overcast conditions between 8:00 and 11:00 am in order to avoid sun streaks. The camera lens was positioned just below the level at which our leaves were selected (i.e. at around 60% of the tree height). Therefore, full sun conditions do not represent 100% canopy openness as they represent leaf-level shade, which takes into account self-shading. For analysis, photographs were then converted into binary images using SideLook 1.1.01 (Nobis & Hunziker, 2005), and binary images were then analyzed using Gap Light Analyser v. 2.0 imaging software (Frazer et al., 1999) to estimate canopy openness. Canopy openness was used to verify our categorical shade treatment designations (monoculture, managed shade and full
Table 1. Mean and standard error of soil inorganic N and P ($n = 105$), and total soil N and C ($n = 35$) across fertilization treatments. F and $p$-values are provided. A Tukey’s post-hoc test of multiple comparisons is denoted by superscripts a, b and c, where same letters indicate no significant difference between treatments (where $p \leq 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>50 kg N ha$^{-1}$yr$^{-1}$</th>
<th>110 kg N ha$^{-1}$yr$^{-1}$</th>
<th>170 kg N ha$^{-1}$yr$^{-1}$</th>
<th>230 kg N ha$^{-1}$yr$^{-1}$</th>
<th>F (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil Inorganic N (mg kg$^{-1}$)</td>
<td>14.15$^a$ ±1.29</td>
<td>18.28$^b$ ±0.84</td>
<td>18.15$^b$ ±1.29</td>
<td>25.84$^c$ ±1.74</td>
<td>13.34 (p≤0.0001)</td>
</tr>
<tr>
<td>Soil Inorganic P (mg kg$^{-1}$)</td>
<td>21.40$^{ab}$ ±0.74</td>
<td>19.82$^a$ ±0.79</td>
<td>27.31$^c$ ±1.17</td>
<td>22.93$^b$ ±1.00</td>
<td>12.49 (p≤0.0001)</td>
</tr>
<tr>
<td>Total Soil N (mg g$^{-1}$)</td>
<td>8.79$^a$ ±0.22</td>
<td>9.22$^a$ ±0.34</td>
<td>10.35$^a$ ±0.43</td>
<td>7.51$^b$ ±0.13</td>
<td>6.84 (p≤0.0001)</td>
</tr>
<tr>
<td>Total Soil C (mg g$^{-1}$)</td>
<td>85.83$^a$ ±1.90</td>
<td>88.79$^{ab}$ ±3.21</td>
<td>104.74$^c$ ±3.87</td>
<td>74.53$^b$ ±1.16</td>
<td>10.78 (p≤0.0001)</td>
</tr>
</tbody>
</table>
Table 2. Mean and standard errors \((n = 105)\) of canopy openness and soil moisture across three shade treatments. \(F\) and \(p\)-values are provided. A Tukey’s post-hoc test of multiple comparisons is denoted by superscripts a, b and c, where same letters indicate no significant difference between treatments (where \(p \leq 0.05\)).

<table>
<thead>
<tr>
<th></th>
<th>Full Shade</th>
<th>Managed Shade</th>
<th>Monoculture</th>
<th>(F \text{ (}p\text{-value)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canopy Openness (%)</td>
<td>28.36(^a) (\pm) 0.74</td>
<td>40.17(^b) (\pm) 1.00</td>
<td>49.51(^c) (\pm) 0.69</td>
<td>160.71(^{\text{p}\leq0.0001})</td>
</tr>
<tr>
<td>Soil Moisture (%)</td>
<td>78.6(^a) (\pm) 1.4</td>
<td>75.6(^a) (\pm) 1.1</td>
<td>79.8(^a) (\pm) 1.5</td>
<td>2.28(^{\text{p}=0.104})</td>
</tr>
</tbody>
</table>
shade) and was significantly different ($p<0.001$) among all three shade levels thus confirming our shade designations (Table 2).

### 3.4 Trait analysis

#### 3.4.1 Aboveground functional traits

Leaf trait sampling was performed following Perez-Harguindeguy et al. (2013). Three leaves per tree were chosen for analysis ($n = 315$), which were young and fully expanded, with no visible signs of damage, located at 60% of the tree height, and taken from reproductive branches (branches which had visible cherry production).

Using a portable photosynthesis system (LI-COR 6400 XT, LI-COR biosciences, Nebraska, USA) we measured light saturated photosynthesis ($A_{\text{sat}}$, µmol CO$_2$ m$^{-2}$ s$^{-1}$) and dark respiration ($R$, µmol CO$_2$ m$^{-2}$ s$^{-1}$). Measurements on individual leaves were taken between 6 and 8 am to avoid midday stomatal closure (Pérez-Harguindeguy et al., 2013). For all measurements, CO$_2$ reference was set to 400 µmol mol$^{-1}$, leaf temperature to 25°C, a constant flow of 400 µmol s$^{-1}$ and humidity was maintained at 50-70%. $A_{\text{sat}}$ was measured first at 1500 µmol m$^{-2}$s$^{-1}$ followed by $R$ at 0 µmol m$^{-2}$s$^{-1}$ ph. Measurements were taken once photosynthetic rates stabilized when clamping onto a new leaf or changing from light saturated to dark respiration (~20 seconds). All $A_{\text{sat}}$ and $R$ measurements were taken as the mean value of five replicate measurements taken 20 seconds apart. Once $A_{\text{sat}}$ and $R$ measurements were complete, the leaf, including the petiole, was collected for chemical and morphological trait analysis.

Immediately following collection, leaf thickness was determined using electronic calipers, taking care to avoid major veins and sampling in the same place for each subsequent leaf. An average from three measurements on the leaf determined leaf thickness (mm). Leaves were then wrapped in moist paper towel and placed into a plastic bag, to avoid dehydration and shrinkage. Once in the lab, leaves were weighed to the precision of 2 decimal places, to determine wet leaf mass. Leaves were then placed on a white board and photographed with a scale bar for analysis in ImageJ V 1.45 software (Wayne Rasband, National Institute of Health, USA) to determine leaf area (LA, cm$^2$). A known scale was set in ImageJ, using the scale bar located in each photograph. This corresponded to a known amount of pixels per unit length which was used to determine LA.
Finally, to obtain dry mass (g), leaves were dried in the oven at 60˚C for a minimum of 24 hours. Using LA and leaf dry mass, specific leaf area (SLA = LA / dry mass of leaf) and leaf mass per area (LMA = 1/SLA) were determined. Using leaf dry mass and leaf wet mass, leaf dry matter content (LDMC = dry mass/wet mass) was also calculated.

Leaf nitrogen concentration (LNC) was analyzed using a LECO CN628 (LECO Corporation, Minnesota, USA) on homogenized dried leaf material (with the petiole removed). Dried leaves were ground in a Retch ball mill (Retsch, Düsseldorf, Germany) and approximately 0.1-0.15 g of sample was weighed and placed into tin foil capsules. Samples were then analyzed on the LECO CN628 for LNC (mg g⁻¹).

3.4.2 Belowground functional traits

Roots were sampled simultaneously with soil sampling (n = 105). One soil core was taken per tree and all available fine roots (<2mm diameter) were sieved and removed. Roots were washed in order to remove all soil and other debris, and processed with WinRHIZO (Reagent Instruments Inc., Quebec) imaging software to determine root diameter (D) and total root length (m). Roots were then dried at 60˚C for 24 hours to determine dry mass (g). Using total root length and dry mass of roots SRL (SRL = total root length / dry root mass) was determined.

3.5 Statistical analysis

Statistical analyses were performed using both SPSS Statistics (SPSS Inc, Illinois, USA) and R statistical analysis software v. 3.2.2 (The R Foundation for Statistical Computing, Vienna, Austria). First, all trait and environmental data was tested for normality using Shapiro-Wilks test. Where environmental and trait data was not normally distributed (namely soil inorganic N, soil inorganic P, total soil N, total soil C and SLA, LMA, LA, leaf thickness, LDMC, SRL, D), log-transformed data was used in analysis. In order to ensure that categorical management treatments resulted in actual environmental variation, I first used a one-way analysis of variance (ANOVA) to test for differences in canopy openness among shade treatments (Table 2) as well as inorganic soil N and P among fertilization treatment (Table 1). Gravimetric soil moisture had no difference between treatments and therefore was not considered a controlling variable for trait expression (p=0.104).
To evaluate both the extent and causes of intraspecific variation in coffee traits, I first used one-way ANOVA in order to evaluate how above and belowground traits vary as a function of both shade and fertilizer treatment, as well as shade-by-fertilizer interactions. This analysis then informed my subsequent analysis, which was designed to evaluate the extent of intraspecific trait variation. I then used a multi-step procedure. For this analysis, I first calculated coefficients of variation (cv) for each trait across the entire dataset. Then, based on the one-way ANOVA results above, I subset all trait data according to the shade and fertilization treatments which had significant differences ($p<0.05$). For each subset of data, I calculated the cv for each trait. I then used a bootstrapping with replacement procedure to generate 999 randomized trait datasets, using the entire dataset ($n=315$ for leaves or $n=105$ for roots). Within each randomized dataset, I then calculated a cv for each trait in order to generate a null model of cv values. I then interpreted all observed subset cv values which fell above the 95% confidence limits of the randomized distribution, as having more intraspecific trait variation as a result of the corresponding treatment. All cv value which fell below the 95% confidence limits of the randomized distribution had less intraspecific trait variation as a result of the corresponding treatment. I then used a standardized major axis regression in order to evaluate patterns of bivariate covariation among all coffee leaf and root traits.
Table 3. List of measured coffee plant and environmental variables with corresponding abbreviations and units.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Abbreviation</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Environmental Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inorganic soil nitrogen</td>
<td>Available soil N</td>
<td>mg kg(^{-1})</td>
</tr>
<tr>
<td>Inorganic soil phosphorus</td>
<td>Available soil P</td>
<td>mg kg(^{-1})</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>Soil moisture</td>
<td>%</td>
</tr>
<tr>
<td>Total soil nitrogen</td>
<td>Total soil N</td>
<td>mg g(^{-1})</td>
</tr>
<tr>
<td>Total soil carbon</td>
<td>Total soil C</td>
<td>mg g(^{-1})</td>
</tr>
<tr>
<td>Canopy openness</td>
<td>Canopy openness</td>
<td>%</td>
</tr>
<tr>
<td><strong>Aboveground Physiological Traits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area based light saturated photosynthesis</td>
<td>(A_{\text{sat}})</td>
<td>(\mu)mol CO(_2) m(^{-2}) s(^{-1})</td>
</tr>
<tr>
<td>Mass-based light-saturated photosynthetic rate</td>
<td>(A_{\text{mass}})</td>
<td>(\mu)mol CO(_2) g(^{-1}) s(^{-1})</td>
</tr>
<tr>
<td>Mass-based dark respiration</td>
<td>(R_{\text{mass}})</td>
<td>(\mu)mol CO(_2) g(^{-1}) s(^{-1})</td>
</tr>
<tr>
<td><strong>Aboveground Morphological Traits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific leaf area</td>
<td>SLA</td>
<td>mm(^2) mg(^{-1})</td>
</tr>
<tr>
<td>Leaf area</td>
<td>LA</td>
<td>cm(^2)</td>
</tr>
<tr>
<td>Leaf dry matter content</td>
<td>LDMC</td>
<td>mg g(^{-1})</td>
</tr>
<tr>
<td>Leaf mass per area</td>
<td>LMA</td>
<td>mg mm(^{-2})</td>
</tr>
<tr>
<td>Leaf thickness</td>
<td>Leaf thickness</td>
<td>mm</td>
</tr>
<tr>
<td>Leaf nitrogen concentration</td>
<td>LNC</td>
<td>mg g(^{-1})</td>
</tr>
<tr>
<td><strong>Belowground Morphological Traits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific root length</td>
<td>SRL</td>
<td>m g(^{-1})</td>
</tr>
<tr>
<td>Average root diameter</td>
<td>D</td>
<td>mm</td>
</tr>
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</table>
Chapter 4
Results

4.1 Covariance in functional traits

Leaf and root traits co-varied in a similar way as proposed by the LES (Table 4). $A_{\text{mass}}$ was significantly positively correlated with log-leaf thickness ($r^2=0.026; p=0.002$), log-LA ($r^2=0.026; p=0.002$) and $A_{\text{sat}}$ ($r^2=0.83; p<0.001$) and significantly negatively correlated with log-LMA ($r^2=0.020; p=0.007$) (Fig. 2). $A_{\text{sat}}$ was significantly positive correlated with log-LMA ($r^2=0.026; p=0.002$) and log-LA ($r^2=0.026; p=0.002$) and $A_{\text{sat}}$ ($r^2=0.83; p<0.001$) and significantly negatively correlated with log-LMA ($r^2=0.026; p=0.002$) and log-leaf thickness ($r^2=0.24; p<0.001$) (Fig. 3) while $R_{\text{mass}}$ was significantly negatively correlated with log-LMA ($r^2=0.199; p<0.001$), log-LA ($r^2=0.02; p=0.005$), log-LDMC ($r^2=0.089; p<0.001$) and log-leaf thickness ($r^2=0.176; p<0.001$) (Fig. 4).

$LNC$ was significantly positive correlated with $R_{\text{mass}}$ ($r^2=0.25; p<0.001$) and significantly negatively correlated with log-LMA ($r^2=0.28; p<0.001$), log-LA ($r^2=0.032; p=0.04$), log-LDMC ($r^2=0.194; p<0.001$), log-leaf thickness ($r^2=0.176; p<0.001$) and $A_{\text{sat}}$ ($r^2=0.035; p=0.03$) (Fig. 5). Log-leaf thickness was significantly positively correlated with log-LMA ($r^2=0.25; p<0.001$), log-LA ($r^2=0.126; p<0.001$) and log-LDMC ($r^2=0.267; p<0.001$) (Fig. 6). Log-LDMC had a significantly positive correlation with log-LMA ($r^2=0.51; p<0.001$) (Fig. 7). $Log-D$ was significantly negatively correlated with log-SRL ($r^2=0.172; p<0.001$) and $LNC$ ($r^2=0.091; p<0.001$) (Fig. 8).

4.2 Plant functional trait variation among treatments

There was a significant difference among all leaf-level traits across shade treatments ($p<0.05$) with the exception of LNC ($p=0.220$) (Table 5). Mean $A_{\text{sat}}$ and $A_{\text{mass}}$ were highest under the lowest shade conditions (monoculture and managed shade, 6.81 ±0.17 (µmol CO$_2$ m$^{-2}$ s$^{-1}$), 0.12 ±0.003 (µmol CO$_2$ g$^{-1}$ s$^{-1}$) and the lowest mean values occurring under full shade (5.26 ±0.17 µmol CO$_2$ m$^{-2}$ s$^{-1}$, 0.097 ±0.003 µmol CO$_2$ g$^{-1}$ s$^{-1}$) (Fig. 9). $R_{\text{mass}}$ showed the opposite, with the highest mean value occurring in the full shade condition (-0.038 ±0.001 µmol CO$_2$ g$^{-1}$ s$^{-1}$) and the lowest mean values occurring in the managed shade treatment (-0.04 ±0.002 µmol CO$_2$ g$^{-1}$ s$^{-1}$). Average LA was highest in full shade conditions (46.89 ±1.53 cm$^2$) followed by managed shade
Table 4. Bivariate relationships among eight leaf traits (where $n = 315$, except for LNC where $n = 105$) and two root traits (where $n = 105$). The upper right section of the matrix represents the slopes and associated 95% confidence intervals for a given relationship, based on a standardized major axis regression analysis. Model $r^2$ and one-tailed $p$-values (in brackets) for each bivariate model are presented in the lower left section of the matrix, with significant relationships ($p \leq 0.05$) highlighted in bold.

<table>
<thead>
<tr>
<th>log-LMA</th>
<th>log-LA</th>
<th>log-LDMC</th>
<th>log-leaf thickness</th>
<th>$A_{\text{sat}}$</th>
<th>$A_{\text{mass}}$</th>
<th>$R_{\text{mass}}$</th>
<th>LNC</th>
<th>log-SRL</th>
<th>log-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>log-LMA</td>
<td>-</td>
<td>-0.019 (0.07)</td>
<td>0.06 (0.4)</td>
<td>0.37 (0.4)</td>
<td>0.0014 (0.0013)</td>
<td>-0.083 (-0.07)</td>
<td>-0.19 (-0.07)</td>
<td>-0.0006 (-0.0005)</td>
<td>0.02 (0.018)</td>
</tr>
<tr>
<td>log-LA</td>
<td>0.008 (0.065)</td>
<td>-</td>
<td>-3.25 (-2.9)</td>
<td>19.15 (21.31)</td>
<td>0.07 (0.08)</td>
<td>4.27 (3.82, 4.78)</td>
<td>-9.91 (-8.85)</td>
<td>-0.03 (-0.02)</td>
<td>1.01 (-3.39)</td>
</tr>
<tr>
<td>log-LDMC</td>
<td>0.51 (0.001)</td>
<td>0.005 (0.11)</td>
<td>-</td>
<td>5.89 (6.48)</td>
<td>0.023 (0.03)</td>
<td>1.31 (1.17, 1.47)</td>
<td>-3.05 (-2.7)</td>
<td>-0.009 (-0.008)</td>
<td>-0.30 (-0.28)</td>
</tr>
<tr>
<td>log-leaf thickness</td>
<td>0.25 (0.001)</td>
<td>0.126 (0.02)</td>
<td>0.267 (0.001)</td>
<td>-</td>
<td>0.004 (0.0035, 0.0043)</td>
<td>0.22 (0.25)</td>
<td>-0.52 (-0.47)</td>
<td>-0.0015 (-0.0013)</td>
<td>0.052 (0.06)</td>
</tr>
<tr>
<td>$A_{\text{sat}}$</td>
<td>0.07 (0.001)</td>
<td>0.014 (0.02)</td>
<td>0.093 (0.001)</td>
<td>0.24 (0.001)</td>
<td>-</td>
<td>57.5 (54.9, 60.3)</td>
<td>-133.4 (-119.1)</td>
<td>-0.44 (-0.37)</td>
<td>13.5 (-15.1)</td>
</tr>
<tr>
<td>$A_{\text{mass}}$</td>
<td>0.020 (0.007)</td>
<td>0.026 (0.002)</td>
<td>5.4e-07 (0.49)</td>
<td>0.086 (0.001)</td>
<td>0.83 (0.001)</td>
<td>-</td>
<td>2.32 (2.1, 2.6)</td>
<td>0.007 (0.009)</td>
<td>0.237 (0.027)</td>
</tr>
<tr>
<td>$R_{\text{mass}}$</td>
<td>0.199 (0.001)</td>
<td>0.02 (0.005)</td>
<td>0.089 (0.001)</td>
<td>0.176 (0.001)</td>
<td>0.009 (0.053)</td>
<td>0.009 (0.053)</td>
<td>-</td>
<td>0.003 (0.002, 0.003)</td>
<td>-4.97 (-5.56, -4.45)</td>
</tr>
<tr>
<td>LNC</td>
<td>0.28 (0.001)</td>
<td>0.032 (0.04)</td>
<td>0.194 (0.001)</td>
<td>0.176 (0.001)</td>
<td>0.035 (0.03)</td>
<td>0.0004 (0.042)</td>
<td>0.25 (&lt;0.001)</td>
<td>-</td>
<td>-33 (-40.1, -27.1)</td>
</tr>
<tr>
<td>log-SRL</td>
<td>0.004 (0.149)</td>
<td>0.014 (0.021)</td>
<td>0.001 (0.278)</td>
<td>0.007 (0.06)</td>
<td>0.0006 (0.333)</td>
<td>0.0008 (0.314)</td>
<td>0.0007 (0.318)</td>
<td>0.002 (0.33)</td>
<td>-</td>
</tr>
<tr>
<td>log-D</td>
<td>0.005 (0.12)</td>
<td>0.002 (0.252)</td>
<td>0.012 (0.289)</td>
<td>0.0001 (0.42)</td>
<td>0.0002 (0.41)</td>
<td>0.0002 (0.22)</td>
<td>0.02 (0.008)</td>
<td>0.091 (&lt;0.001)</td>
<td>0.172 (&lt;0.001)</td>
</tr>
</tbody>
</table>
**Figure 2.** Significant bivariate relationships (standardized major axis model) between mass-based light-saturated photosynthetic rate ($A_{\text{mass}}$, $\mu$mol CO$_2$ g$^{-1}$ s$^{-1}$) and (A) leaf area (log-LA, mm$^2$); (B) leaf thickness (log-leaf thickness, mm); (C) photosynthesis under saturating irradiance ($A_{\text{sat}}$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$); and (D) leaf mass per area (log-LMA, mg mm$^{-2}$) across all shade and fertilization treatments. Standardized major axis regression line also presented. Refer to Table 4 for all coefficients of determination ($r^2$) and significance ($p$-value), with corresponding slope and 95% confidence intervals.
Figure 3. Significant bivariate relationships (standardized major axis model) between photosynthesis under saturating irradiance \( A_{\text{sat}, \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}} \) and (A) leaf mass per area (log-LMA, mg mm\(^{-2}\)); (B) leaf dry matter content (log-LDMC, mg g\(^{-1}\)); (C) leaf area (log-LA, mm\(^2\)); and (D) leaf thickness (log-leaf thickness, mm); across all shade and fertilization treatments. Standardized major axis regression line also presented. Refer to Table 4 for all coefficients of determination \((r^2)\) and significance \((p\text{-value})\), with corresponding slope and 95\% confidence intervals.
Figure 4. Significant bivariate relationships (standardized major axis model) between mass-based dark respiration ($R_{mass}$, $\mu$mol CO$_2$ g$^{-1}$s$^{-1}$) and (A) leaf mass per area (log-LMA, mg mm$^{-2}$); (B) leaf area (log-LA, cm$^2$); (C) leaf dry matter content (log-LDMC, mg g$^{-1}$); and (D) leaf thickness (log-leaf thickness, mm); across all shade and fertilization treatments. Standardized major axis regression line also presented. Refer to Table 4 for all coefficients of determination ($r^2$) and significance ($p$-value), with corresponding slope and 95% confidence intervals.
Figure 5. Significant bivariate relationships (standardized major axis model) between leaf nitrogen concentration (LNC, mg g\(^{-1}\)) and (A) mass-based dark respiration (\(R_{\text{mass}}, \mu\text{mol CO}_2 \text{ g}^{-1}\text{s}^{-1}\)); (B) photosynthesis under saturating irradiance (\(A_{\text{sat}}, \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}\)); (C) leaf area (log-LA, cm\(^2\)); (D) leaf dry matter content (log-LDMC, mg g\(^{-1}\)); (E) leaf thickness (log-leaf thickness\(^h\), mm); and (F) leaf mass per area (log-LMA, mg mm\(^{-2}\)) across all shade and fertilization treatments. Standardized major axis regression line also presented. Refer to Table 4 for all coefficients of determination (\(r^2\)) and significance (\(p\)-value), with corresponding slope and 95% confidence intervals.
Figure 6. Significant bivariate relationships (standardized major axis model) between leaf thickness (log-leaf thickness, mm) and (A) leaf mass per area (log-LMA, mg mm$^{-2}$); (B) leaf area (log-LA, cm$^2$); and (C) leaf dry matter content (log-LDMC, mg g$^{-1}$) across all shade and fertilization treatments. Standardized major axis regression line also presented. Refer to Table 4 for all coefficients of determination ($r^2$) and significance ($p$-value), with corresponding slope and 95% confidence intervals.
**Figure 7.** Significant bivariate relationships (standardized major axis model) between leaf dry matter content (log-LDMC, mg g$^{-1}$) and leaf mass per area (log-LMA, mg mm$^{-2}$) across all shade and fertilization treatments. Standardized major axis regression line also presented. Refer to Table 4 for all coefficients of determination ($r^2$) and significance ($p$-value), with corresponding slope and 95% confidence intervals.
Figure 8. Significant bivariate relationships (standardized major axis model) between average root diameter (log-D, mm) and (A) specific root length (log-SRL, m g\(^{-1}\)); and (B) leaf nitrogen concentration (LNC, mg g\(^{-1}\)) across all shade and fertilization treatments. Standardized major axis regression line also presented. Refer to Table 4 for all coefficients of determination (\(r^2\)) and significance (\(p\)-value), with corresponding slope and 95% confidence intervals.
(35.78 ±1.26 cm²) and monoculture plots (29.87 ±1.20 cm²). LMA was inversely related to shade, with the highest mean values occurring in the monoculture plots (0.058 ±0.001 mg mm⁻²) and the lowest mean values occurring in the full shade plots (0.055 ±0.001 mg mm⁻²) (Fig. 10). SLA showed the opposite with the highest average values in the full shade plots (18.45 ±0.22 mm² mg⁻¹) and lowest in the monoculture plots (17.43 ±0.21 mm² mg⁻¹) (Fig. 10). LDMC and leaf thickness were also inversely related to shade, with the highest mean values occurring in the monoculture plot (240.08 ±1.89 mg g⁻¹, 0.24 ±0.002 mm) followed by the managed shade (235.57 ±2.15 mg g⁻¹, 0.24 ±0.002 mm), with the smallest mean values for each trait occurring in the full shade conditions (222.46 ±2.26 mg g⁻¹, 0.23 ±0.002 mm). Average root diameter was also significantly different across shade treatment (p=0.04), with the largest mean root diameter occurring in the full shade treatments (0.89 ±0.03 mm) and the smallest occurring in the managed shade treatments (0.82 ±0.01 mm) (Fig. 11).

There was a significant relationship between root and leaf-level traits and fertilizer treatments (p<0.05) (Table 6). Average $A_{sat}$ was highest in 230 kg N ha⁻¹yr⁻¹ treatments (6.80 ±0.19 µmol CO₂ m⁻² s⁻¹) and lowest in the 170 kg N ha⁻¹yr⁻¹ treatments (4.96 ±0.19 µmol CO₂ m⁻² s⁻¹) while $A_{mass}$ had the highest average occurring in the 230 kg N ha⁻¹yr⁻¹ and 110 kg N ha⁻¹yr⁻¹ treatments (0.12 ±0.003 µmol CO₂ g⁻¹ s⁻¹) and lowest values occurring in the 170 kg N ha⁻¹yr⁻¹ treatments (0.09 ±0.003 µmol CO₂ g⁻¹ s⁻¹) (Fig. 12). $R_{mass}$ had the highest rates of average respiration in the 50 kg N ha⁻¹yr⁻¹ treatments (-0.03 ±0.002 µmol CO₂ g⁻¹ s⁻¹) and the lowest in the 110 kg N ha⁻¹yr⁻¹ treatments (-0.05 ±0.002 µmol CO₂ g⁻¹ s⁻¹).

Average SLA was highest in the 110 kg N ha⁻¹yr⁻¹ treatments (18.80 ±0.24 mm²mg⁻¹) and lowest in the 170 kg N ha⁻¹yr⁻¹ treatments (17.48 ±0.20 mm²mg⁻¹), while average LMA was lowest in the 110 kg N ha⁻¹yr⁻¹ (0.054 ±0.001 mg mm⁻²) and highest in the 170 kg N ha⁻¹yr⁻¹ (0.058a ±0.001 mg mm⁻²) (Fig. 13). Average LDMC was highest in the 170 kg N ha⁻¹yr⁻¹ treatments (240.21 ±2.91 mg g⁻¹) and lowest in the 110 kg N ha⁻¹yr⁻¹ and 230 kg N ha⁻¹yr⁻¹ treatments (229.27 ±2.20 mg g⁻¹ and 229.68 ±2.48 mg g⁻¹). SRL had the highest mean length occurring under the lowest fertilization condition (9.55 ±0.41 m g⁻¹) and the lowest mean length occurring in the highest fertilization condition (8.42 ±0.36 m g⁻¹) (Fig. 14). Average root diameter had a less defined relationship with the highest average occurring under the 110 kg N ha⁻¹yr⁻¹ fertilization
**Table 5.** Mean and standard error of leaf (where \( n = 315 \), with the exception of LNC where \( n = 105 \)) and root (where \( n = 105 \)) traits across three shade treatments. \( F \) and \( p \)-values are provided. A Tukey’s post-hoc test of multiple comparisons is denoted by superscripts a, b and c, where same letters indicate no significant difference between treatments (where \( p \leq 0.05 \)).

<table>
<thead>
<tr>
<th>Shade Treatment</th>
<th>Monoculture</th>
<th>Managed Shade</th>
<th>Full Shade</th>
<th>( F ) (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA (mm²·mg⁻¹)</td>
<td>17.43 ±0.21</td>
<td>18.18b ±0.19</td>
<td>18.45b ±0.22</td>
<td>7.04 (0.001)</td>
</tr>
<tr>
<td>LA (cm²)</td>
<td>29.87 ±1.19</td>
<td>35.78b ±1.26</td>
<td>46.89c ±1.53</td>
<td>36.44 (p≤0.0001)</td>
</tr>
<tr>
<td>LMA (mg mm⁻²)</td>
<td>0.058 ±0.001</td>
<td>0.056b ±0.001</td>
<td>0.055b ±0.001</td>
<td>7.187 (0.001)</td>
</tr>
<tr>
<td>LDMC (mg g⁻¹)</td>
<td>240.08 ±1.89</td>
<td>235.57a ±2.15</td>
<td>222.46b ±2.26</td>
<td>20.585 (p≤0.0001)</td>
</tr>
<tr>
<td>Leaf thickness (mm)</td>
<td>0.24a ±0.002</td>
<td>0.24a ±0.002</td>
<td>0.23b ±0.002</td>
<td>4.840 (0.009)</td>
</tr>
<tr>
<td>LNC (mg g⁻¹)</td>
<td>33.88a ±0.71</td>
<td>34.38a ±0.77</td>
<td>32.52a ±0.82</td>
<td>1.539 (0.220)</td>
</tr>
<tr>
<td>( A_{\text{sat}} ) (µmol CO₂ m⁻² s⁻¹)</td>
<td>6.81a ±0.17</td>
<td>6.48a ±0.17</td>
<td>5.26b ±0.17</td>
<td>22.782 (p≤0.0001)</td>
</tr>
<tr>
<td>( A_{\text{mass}} ) (µmol CO₂ g⁻¹ s⁻¹)</td>
<td>0.12a ±0.003</td>
<td>0.12a ±0.003</td>
<td>0.097b ±0.003</td>
<td>13.708 (p≤0.0001)</td>
</tr>
<tr>
<td>( R_{\text{mass}} ) (µmol CO₂ g⁻¹ s⁻¹)</td>
<td>-0.04a ±0.002</td>
<td>-0.04a ±0.002</td>
<td>-0.038b ±0.001</td>
<td>3.870 (0.022)</td>
</tr>
<tr>
<td>SRL (m g⁻¹)</td>
<td>8.61a ±0.26</td>
<td>9.24a ±0.34</td>
<td>9.09a ±0.30</td>
<td>1.069 (0.345)</td>
</tr>
<tr>
<td>D (mm)</td>
<td>0.85ab ±0.02</td>
<td>0.82a ±0.01</td>
<td>0.89b ±0.03</td>
<td>3.224 (0.041)</td>
</tr>
</tbody>
</table>
Figure 9. Probability distribution function imposed over histograms of (A) mass-based light-saturated photosynthetic rate ($A_{\text{mass}}, \mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) and (B) photosynthesis under saturating irradiance ($A_{\text{sat}}, \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) ($n = 315$), with normal distribution curves for each shade treatment (full shade, managed shade and monoculture), where the peak of the curve is the mean trait value. Refer to Table 7 for overall mean and Table 5 for mean trait values among each shade treatment.
Figure 10. Probability distribution function imposed over histograms of (A) leaf mass per area (LMA, mg mm$^{-2}$) and (B) specific leaf area (SLA, mm$^2$ mg$^{-1}$) ($n = 315$), with log-normal distribution curves for each shade treatment (full shade, managed shade and monoculture), where the peak of the curve is the mean trait value. Refer to Table 7 for overall mean and Table 5 for mean trait values among each shade treatment.
Figure 11. Probability distribution function imposed over histograms of (A) specific root length (SRL, m g\(^{-1}\)) and (B) average root diameter (D, mm) (\(n = 105\)), with log-normal distribution curves for each shade treatment (full shade, managed shade and monoculture), where the peak of the curve is the mean trait value. Refer to Table 7 for overall mean and Table 5 for mean trait values among each shade treatment.
Table 6. Mean and standard error of leaf (where \( n = 315 \), with the exception of LNC where \( n = 105 \)) and root (where \( n = 105 \)) traits across four nutrient treatments. \( F \) and \( p \)-values are provided. A Tukey’s post-hoc test of multiple comparisons is denoted by superscripts a, b and c, where same letters indicate no significant difference between treatments (where \( p \leq 0.05 \)).

<table>
<thead>
<tr>
<th>Fertilizer Treatment</th>
<th>[\text{50 kg N ha}^{-1}\text{y}^{-1}]</th>
<th>[\text{110 kg N ha}^{-1}\text{y}^{-1}]</th>
<th>[\text{170 kg N ha}^{-1}\text{y}^{-1}]</th>
<th>[\text{230 kg N ha}^{-1}\text{y}^{-1}]</th>
<th>( F ) (( p )-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA (mm(^2)mg(^{-1}))</td>
<td>17.58(^{a}) ±0.24</td>
<td>18.80(^{b}) ±0.24</td>
<td>17.48(^{a}) ±0.20</td>
<td>18.12(^{ab}) ±0.26</td>
<td>6.828 (( p \leq 0.0001 ))</td>
</tr>
<tr>
<td>LA (cm(^2))</td>
<td>32.32(^{a}) ±1.96</td>
<td>42.19(^{a}) ±1.46</td>
<td>39.16(^{a}) ±1.60</td>
<td>39.16(^{a}) ±1.60</td>
<td>2.391 (0.069)</td>
</tr>
<tr>
<td>LMA (mg mm(^{-2}))</td>
<td>0.058(^{a}) ±0.001</td>
<td>0.054(^{b}) ±0.001</td>
<td>0.058(^{a}) ±0.001</td>
<td>0.056(^{ab}) ±0.001</td>
<td>6.304 (( p \leq 0.0001 ))</td>
</tr>
<tr>
<td>LDMC (mg g(^{-1}))</td>
<td>232.81(^{ab}) ±2.38</td>
<td>229.27(^{a}) ±2.20</td>
<td>240.21(^{b}) ±2.91</td>
<td>229.68(^{a}) ±2.48</td>
<td>3.955 (0.009)</td>
</tr>
<tr>
<td>Leaf thickness (mm)</td>
<td>0.24(^{a}) ±0.002</td>
<td>0.24(^{a}) ±0.002</td>
<td>0.24(^{a}) ±0.002</td>
<td>0.24(^{a}) ±0.002</td>
<td>1.424 (0.236)</td>
</tr>
<tr>
<td>LNC (mg g(^{-1}))</td>
<td>32.56(^{a}) ±0.86</td>
<td>32.56(^{a}) ±0.86</td>
<td>34.97(^{a}) ±0.92</td>
<td>33.58(^{a}) ±0.73</td>
<td>1.322 (0.271)</td>
</tr>
<tr>
<td>( A_{\text{sat}} ) (μmol CO(_2) m(^{-2}) s(^{-1}))</td>
<td>6.69(^{a}) ±0.22</td>
<td>6.44(^{a}) ±0.16</td>
<td>4.96(^{b}) ±0.19</td>
<td>6.80(^{a}) ±0.19</td>
<td>20.55 (( p \leq 0.0001 ))</td>
</tr>
<tr>
<td>( A_{\text{mass}} ) (μmol CO(_2) g(^{-1}) s(^{-1}))</td>
<td>0.11(^{a}) ±0.004</td>
<td>0.12(^{a}) ±0.003</td>
<td>0.09(^{b}) ±0.003</td>
<td>0.12(^{a}) ±0.003</td>
<td>20.20 (( p \leq 0.0001 ))</td>
</tr>
<tr>
<td>( R_{\text{mass}} ) (μmol CO(_2) g(^{-1}) s(^{-1}))</td>
<td>-0.03(^{a}) ±0.002</td>
<td>-0.05(^{b}) ±0.002</td>
<td>-0.04(^{a}) ±0.002</td>
<td>-0.04(^{ab}) ±0.002</td>
<td>3.162 (0.025)</td>
</tr>
<tr>
<td>SRL (m g(^{-1}))</td>
<td>9.55(^{a}) ±0.41</td>
<td>8.94(^{ab}) ±0.39</td>
<td>9.04(^{ab}) ±0.24</td>
<td>8.42(^{ab}) ±0.36</td>
<td>2.705 (0.045)</td>
</tr>
<tr>
<td>D (mm)</td>
<td>0.83(^{a}) ±0.02</td>
<td>0.93(^{b}) ±0.03</td>
<td>0.81(^{a}) ±0.01</td>
<td>0.84(^{a}) ±0.01</td>
<td>5.956 (0.001)</td>
</tr>
</tbody>
</table>
Figure 12. Probability distribution function imposed over histograms of (A) mass-based light-saturated photosynthetic rate ($A_{\text{mass}}$, $\mu$mol CO$_2$ g$^{-1}$ s$^{-1}$) and (B) photosynthesis under saturating irradiance ($A_{\text{sat}}$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) ($n = 315$), with normal distribution curves for each shade treatment (full shade, managed shade and monoculture), where the peak of the curve is the mean trait value. Refer to Table 7 for overall mean and Table 6 for mean trait values among each nutrient treatment.
Figure 13. Probability distribution function imposed over histograms of (A) leaf mass per area (LMA, mg mm$^{-2}$) and (B) specific leaf area (SLA, mm$^2$ mg$^{-1}$) ($n = 315$), with log-normal distribution curves for each shade treatment (full shade, managed shade and monoculture, where the peak of the curve is the mean trait value. Refer to Table 7 for overall mean and Table 6 for mean trait values among each nutrient treatment.
Figure 14. Probability distribution function imposed over histograms of (A) specific root length (SRL, m g⁻¹) and (B) average root diameter (D, mm) \((n = 105)\), with log-normal distribution curves for each shade treatment (full shade, managed shade and monoculture, where the peak of the curve is the mean trait value. Refer to Table 7 for overall mean and Table 6 for mean trait values among each nutrient treatment.
treatments (0.93 ±0.03 mm) and the lowest average occurring under the 170 kg N ha\(^{-1}\)yr\(^{-1}\) treatments (0.81 ±0.01 mm) (Fig. 14).

### 4.3 Intraspecific trait variation

Analysis of intraspecific variation utilized coefficients of variation. Large amounts of variation was seen in physiological leaf traits \(A_{\text{sat}}\) (cv = 29.6), \(A_{\text{mass}}\) (cv = 29.01) and \(R_{\text{mass}}\) (cv = 33.28) as well as in root traits SRL (cv = 34.75) and D (cv = 22.54) and LA (cv = 32.89) (Table 7). Physiological leaf trait variation was largest under full shade for \(A_{\text{sat}}\) (32.68), \(A_{\text{mass}}\) (32.54) and \(R_{\text{mass}}\) (36.26) (Table 8), all higher than the modeled cv 95% confidence intervals. Morphological trait variation under full shade was within the expected 95% confidence limits of the modeled cv. Under managed shade treatments, cvs were lower than the modeled cv in \(A_{\text{sat}}\) (26.79), \(A_{\text{mass}}\) (26.13), \(R_{\text{mass}}\) (28.40), SLA (10.21), LA (24.55), LMA (10.35) and leaf thickness (7.47) (Table 8). Cvs for traits under monocultures were lower for \(A_{\text{sat}}\) (25.13), \(A_{\text{mass}}\) (25.67), LA (27.45) and LDMC (8.18) and but higher for leaf thickness (9.25) as compared to the modeled cv (Table 8).

Under the lowest fertilization treatment, trait variation was within the expected 95% confidence limits of the modeled cv in all leaf and root traits (Table 9). Under the 110 kg N ha\(^{-1}\)yr\(^{-1}\), intraspecific variation was higher than the modeled cv in SRL (38.76) and D (31.47) (Table 9). Under this same nutrient treatment, variation was lower than the modeled cv in LDMC (8.62), \(A_{\text{sat}}\) (22.63) and \(A_{\text{mass}}\) (22.85). Under the 170 kg N ha\(^{-1}\)yr\(^{-1}\), intraspecific variation was higher than the modelled cv in LDMC and \(A_{\text{sat}}\), and lower than the modelled cv in SLA (10.13), LA (10.00), SRL (23.36) and D (10.12). Finally, under the highest fertilization treatment (230 kg N ha\(^{-1}\)yr\(^{-1}\), intraspecific trait variation was lower than the modelled cv in \(A_{\text{mass}}\) (22.92) and D (11.01).
Table 7. Mean and standard error, including the range in values observed for each leaf ($n = 315$) and root ($n = 105$) trait, with corresponding coefficient of variation for leaf and root traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean + Std Error</th>
<th>Range</th>
<th>Overall cv</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA (mm$^2$mg$^{-1}$)</td>
<td>18.02 ±0.12</td>
<td>13.34 - 23.82</td>
<td>11.48</td>
</tr>
<tr>
<td>LA (cm$^2$)</td>
<td>39.24 ±0.75</td>
<td>17.41 - 83.76</td>
<td>32.89</td>
</tr>
<tr>
<td>LMA (mg mm$^{-2}$)</td>
<td>0.06 ±0.0004</td>
<td>0.04 - 0.07</td>
<td>11.47</td>
</tr>
<tr>
<td>LDMC (mg g$^{-1}$)</td>
<td>232.99 ±1.28</td>
<td>165.75 - 324.28</td>
<td>9.72</td>
</tr>
<tr>
<td>Leaf thickness (mm)</td>
<td>0.24 ±0.001</td>
<td>0.18 - 0.29</td>
<td>8.5</td>
</tr>
<tr>
<td>LNC (mg g$^{-1}$)</td>
<td>33.63 ±0.44</td>
<td>22.90 - 42.78</td>
<td>13.37</td>
</tr>
<tr>
<td>$A_{sat}$ ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>6.21 ±0.10</td>
<td>1.88 - 11.70</td>
<td>29.6</td>
</tr>
<tr>
<td>$A_{mass}$ ($\mu$mol CO$_2$ g$^{-1}$ s$^{-1}$)</td>
<td>0.12 ±0.002</td>
<td>0.03 - 0.19</td>
<td>29.01</td>
</tr>
<tr>
<td>$R_{mass}$ ($\mu$mol CO$_2$ g$^{-1}$ s$^{-1}$)</td>
<td>-0.04 ±0.001</td>
<td>-0.08 - -0.01</td>
<td>33.28</td>
</tr>
<tr>
<td>SRL (m g$^{-1}$)</td>
<td>8.97 ±0.18</td>
<td>3.77 - 21.50</td>
<td>34.75</td>
</tr>
<tr>
<td>D(mm)</td>
<td>0.85 ±0.01</td>
<td>0.59 - 1.81</td>
<td>22.54</td>
</tr>
</tbody>
</table>
Table 8. Coefficients of variation for leaf \((n = 315, \text{ with the exception of LNC where } n=105)\) and root \((n = 105)\) traits within each shade treatment designation, as well as the model cv, a modeled distribution of data based on the whole data set, with corresponding 95% confidence limits. Trait-treatment combinations which showed increased (+) or decreased (-) variation are in bold.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SLA (mm²mg⁻¹)</th>
<th>LA (cm²)</th>
<th>LMA (mg mm⁻²)</th>
<th>LDMC (mg g⁻¹)</th>
<th>Leaf thickness (mm)</th>
<th>(A_{\text{sat}}) (µmol CO₂ m⁻² s⁻¹)</th>
<th>(A_{\text{mass}}) (µmol CO₂ g⁻¹ s⁻¹)</th>
<th>(R_{\text{mass}}) (µmol CO₂ g⁻¹ s⁻¹)</th>
<th>D (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Shade</td>
<td>11.77</td>
<td>32.46</td>
<td>11.54</td>
<td>10.11</td>
<td>8.36</td>
<td>32.68 *</td>
<td>32.54 *</td>
<td>36.26 *</td>
<td>29.67 *</td>
</tr>
<tr>
<td>Managed Shade</td>
<td><strong>10.21</strong></td>
<td><strong>24.55</strong></td>
<td><strong>10.35</strong></td>
<td>9.46</td>
<td><strong>7.47</strong></td>
<td><strong>26.79</strong></td>
<td>26.13</td>
<td><strong>28.40</strong></td>
<td>10.81</td>
</tr>
<tr>
<td>Monoculture</td>
<td>11.79</td>
<td>27.45</td>
<td>11.74</td>
<td><strong>8.18</strong></td>
<td><strong>9.25</strong></td>
<td><strong>25.13</strong></td>
<td><strong>25.67</strong></td>
<td>34.49</td>
<td>21.71</td>
</tr>
<tr>
<td>Model cv (95% confidence limits)</td>
<td>11.45 (10.54, 12.36)</td>
<td>32.79 (30.46, 35.12)</td>
<td>11.42 (10.47, 12.36)</td>
<td>9.69 (8.71, 10.66)</td>
<td>8.48 (7.91, 9.04)</td>
<td>29.5 (27.17, 31.84)</td>
<td>28.94 (26.49, 31.38)</td>
<td>33.30 (30.59, 35.99)</td>
<td>22.18 (17.69, 26.66)</td>
</tr>
</tbody>
</table>
Table 9. Coefficients of variation for leaf ($n = 315$, with the exception of LNC where $n=105$) and root ($n = 105$) traits within fertilization treatment designation, as well as the model cv, a modeled distribution of data based on the whole data set, with corresponding 95% confidence limits. Trait-treatment combinations which showed increased (+) or decreased (-) variation are in bold.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SLA (mm$^2$mg$^{-1}$)</th>
<th>LMA (mg mm$^{-2}$)</th>
<th>LDMC (mg g$^{-1}$)</th>
<th>$A_{sat}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>$A_{mass}$ (µmol CO$_2$ g$^{-1}$ s$^{-1}$)</th>
<th>$R_{mass}$ (µmol CO$_2$ g$^{-1}$ s$^{-1}$)</th>
<th>SRL (m g$^{-1}$)</th>
<th>D (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 kg N ha$^{-1}$yr$^{-1}$</td>
<td>10.64</td>
<td>10.71</td>
<td>8.69</td>
<td>28.02</td>
<td>27.69</td>
<td>31.37</td>
<td>36.65</td>
<td>24.58</td>
</tr>
<tr>
<td>110 kg N ha$^{-1}$yr$^{-1}$</td>
<td>11.44</td>
<td>11.68</td>
<td><strong>8.62</strong></td>
<td><strong>22.63</strong></td>
<td><strong>22.85</strong></td>
<td>31.34</td>
<td><strong>38.76</strong></td>
<td><strong>31.47</strong></td>
</tr>
<tr>
<td>170 kg N ha$^{-1}$yr$^{-1}$</td>
<td><strong>10.13</strong></td>
<td><strong>10.00</strong></td>
<td><strong>10.89</strong></td>
<td><strong>33.71</strong></td>
<td>31.02</td>
<td>31.99</td>
<td><strong>23.36</strong></td>
<td><strong>10.12</strong></td>
</tr>
<tr>
<td>230 kg N ha$^{-1}$yr$^{-1}$</td>
<td>12.11</td>
<td>12.28</td>
<td>9.71</td>
<td>27.29</td>
<td><strong>22.92</strong></td>
<td>31.37</td>
<td>37.92</td>
<td><strong>11.01</strong></td>
</tr>
<tr>
<td>Model cv</td>
<td>11.43</td>
<td>11.48</td>
<td>9.69</td>
<td>29.60</td>
<td>28.98</td>
<td>32.68</td>
<td>34.50</td>
<td>22.42</td>
</tr>
<tr>
<td>(95% confidence limits)</td>
<td>(10.49, 12.36)</td>
<td>(10.60, 12.36)</td>
<td>(8.75, 10.65)</td>
<td>(27.29, 31.92)</td>
<td>(26.62, 31.34)</td>
<td>(30.22, 35.15)</td>
<td>(38.69, 38.69)</td>
<td>(18.02, 26.82)</td>
</tr>
</tbody>
</table>
Chapter 5
Discussion

5.1 Covariance in leaf and root traits

The LES describes a generalized pattern of leaf trait covariation, where at the resource acquiring end of the spectrum fast-growing, short-lived species are characterized by low LMA, and high $A_{mass}$, $R_{mass}$ and LNC. At the opposite end of the spectrum, slow-growing, long-lived species characterized by high LMA and low $A_{mass}$, $R_{mass}$ and LNC (Wright et al., 2004). Within species trait covariation is less well documented, although studies have found similar but weaker relationships as those hypothesized by the LES (Gagliardi et al., 2015). I hypothesized that coffee leaf traits would covary following the same patterns predicted by the LES. I found that high LNC corresponded to low LMA and high $R_{mass}$ (Fig. 5), as reported in the LES (Wright et al., 2004). There was also a significantly negative relationship between $A_{mass}$ and LMA (Fig. 2). One LES pattern which was not found, was a significant relationship between LNC and $A_{mass}$. This could have been a result of small sample size, as time constraints forced the sample size of LNC to be smaller than the rest of the leaf trait data ($n=105$ versus $n=315$). The $r^2$ values between $A_{mass}$ and $A_{sat}$ and associated morphological traits (LA, LMA, LDMC) were very weak. For example, between $A_{mass}$ and LMA, the LES reported an $r^2$ value of 0.50 (Wright et al., 2004), however, the $r^2$ in this study was only 0.020 (Table 4). Coefficients of determination were also weaker than values reported by the LES, in a study by Gagliardi et al. (2015), where within species covariation was assessed. This suggests that within species trait correlations are not as predictable or strong as between species correlations.

Specific root length was negatively correlated with average root diameter (Fig. 8) which is consistent with previous studies on root traits (Comas et al., 2009; Prieto et al., 2015; Tobner et al., 2013). When roots traits are placed along an economic scale similar to that proposed by the LES, at the resource acquiring end of the spectrum one would expect to find fast-growing, short-lived species which exhibit high SRL and low D, with the opposite suite of traits for slow-growing, long-lived, resource conserving plants (Bardgett et al., 2014; Ostonen et al., 2007).
5.2 Plant functional traits among treatments

I hypothesized coffee leaf traits would vary systematically across shade treatments, while root functional traits would vary systematically across nutrient treatments. My results support this hypothesis, with some exceptions. Shade had a significant effect on leaf trait expression (Table 5). Mean coffee plant trait values in monoculture versus full shade treatments were significantly different for all leaf level traits, with the exception of LNC. It should be noted that LNC, while not significantly different between treatments, had a range from 22-42 mg g$^{-1}$, which is within the optimal range for coffee growth (Marsh et al., 2006). Plants in the managed shade condition had trait values that were not significantly different from plants in monoculture (LDMC, leaf thickness, $A_{sat}$, $A_{mass}$, $R_{mass}$). The amount of light in the monoculture and managed shade plots were similar (absolute different of 9% canopy openness) which could potentially explain these similar mean trait values across management systems.

A plants’ position along the LES can be used to predict the growth strategies, survival and reproductive success of a plant (Gagliardi et al., 2015; Reich, 2014; Wright et al., 2004). In this study, low levels of light (full shade treatments) promoted a mixture of resource conserving and resource acquiring leaf and root traits. Resource acquiring leaf traits in the full shade conditions consisted of low LMA and high SLA (Fig. 10) and resource acquiring root traits consisted of high SRL (Fig. 11). Resource conserving traits in the full shade conditions consisted of low $A_{mass}$ and $A_{sat}$ (Fig. 9). This result slightly confounds the relationships proposed by the LES, where high SLA would accompany high $A$ values (Wright et al., 2004). Previous work has reported that shade-grown coffee experiences higher $A$ values than full sun plots (Gagliardi et al., 2015), however there are studies which have reported lower $A$ values under shade (DaMatta, 2004; Martins et al., 2014). Martins et al., (2014) suggest $A$ values are limited primarily by stomatal factors. For example, mid-day stomatal closure, which is an outcome of high leaf temperatures and vapour pressure deficits, results in lower $A$ values. Monoculture plots will experience more midday stomatal closure than an agroforestry plot, since the shade from a tree controls and regulates local temperature (Charbonnier et al., 2013). Since my study accounted for midday stomatal closure, by sampling early in the morning when temperatures were low, this may partially explain these results. Another explanation for lower $A$ values under shaded conditions could have been caused by unusually high levels of shade. Franck et al. (2009) report a decrease
in net photosynthesis values of up to 20% when coffee plants experience over 55% shade. Since there was a high amount of shade in the full shade plots (71%), this may explain why there were low A values, rather than the expected high A values which typically accompany low LMA. Finally, coffee plants grown in the full sun will generally show a decrease in photosynthetic capacity, known as photoinhibition, however increased soil nutrients can compensate for this (DaMatta, 2004; Nunes et al., 1993). Therefore, monoculture plots may not be experiencing photoinhibition since there was an ample supply of soil nutrient (Table 1).

My findings also show that leaf trait expression was different across the fertilization treatments (Table 6). Previous work has shown that SLA and LNC increase with increased soil nutrients (Ordoñez et al., 2009) and the same traits decrease in nutrient poor soil (Jager et al., 2015). In this study, the highest values for SLA occurred in the 110 and 230 kg N ha\(^{-1}\) yr\(^{-1}\) plots and the lowest SLA in the 50 and 170 kg N ha\(^{-1}\) yr\(^{-1}\) plots (Fig. 13). The highest values for LMA occurred in the 50 kg N ha\(^{-1}\) yr\(^{-1}\) and 170 kg N ha\(^{-1}\) yr\(^{-1}\) plots, with the lowest LMA occurring in the 110 kg N ha\(^{-1}\) yr\(^{-1}\) plot and 230 kg N ha\(^{-1}\) yr\(^{-1}\) plots (Fig. 13). LNC was not significantly different among nutrient treatments (Table 6). However, in the lowest nutrient condition (50 kg N ha\(^{-1}\) yr\(^{-1}\)) where the conservation of nutrients is likely more important, resource conserving leaf traits including low SLA and high LMA were observed, which is consistent with previous studies on resource conservation strategies and soil fertility (Lavorel et al., 2002; Ordoñez et al., 2009).

As hypothesized, fertilization also had a significant influence on root trait expression (Table 6). The 50 kg N ha\(^{-1}\) yr\(^{-1}\) plots had the highest average SRL, while the 230 kg N ha\(^{-1}\) yr\(^{-1}\) plots had the lowest average SRL (Fig. 14). Further, the 50 kg N ha\(^{-1}\) yr\(^{-1}\) plots had the lowest D, while the 170 kg N ha\(^{-1}\) yr\(^{-1}\) plots had the highest D (Fig. 14). A high SRL would be considered a resource acquiring trait (Bardgett et al., 2014; Ostonen et al., 2007), and therefore, low fertilization treatments promoted resource acquiring root strategies whereas the highest fertilization treatment (230 kg N ha\(^{-1}\) yr\(^{-1}\)) promoted resource conserving root traits. This finding is consistent with studies where higher SRL was reported in low nutrient treatments (Ostonen et al., 2007; Prieto et al., 2015; Trubat et al., 2006), but contradicts studies that report positive (Majdi & Viebke, 2004) or no (Mei et al., 2010; Tobner et al., 2013) relationship between SRL and nutrient input. Tobner et al. (2013) suggested that the response of SRL is highly dependent on the type of fertilizer used, the sampling method (e.g. in-growth cores versus field samples) and the root diameter that
is sampled (0-1mm, <2 mm). Looking at our soil nutrient data, I also see that roots correlated more closely to inorganic soil P, rather than inorganic soil N. Therefore, SRL had a significantly negative relationship with the complete fertilizer formula of 24.5-0-15-5-0.43 (N-P-K₂O-Mg-B), when sampled to a depth of 20 cm in field, with a root diameter of <2 mm.

I found significant correlations among suites of both leaf and root traits, however only a few of these aboveground and belowground traits correlated with one another. Studies have attempted to quantify the relationships between leaf and root functional traits with varying results. Withington et al., (2006) reports significant correlations between SRL and SLA while Tjoelker et al., (2005) did not find significant correlations between leaf and root traits. SRL has a weak, positive correlation with LA, and D had weak, positive correlations with LDMC and weak negative correlations with \( R_{\text{mass}} \) and LNC. In order to understand these correlations, the environmental stimuli associated with leaf and root proliferation need to be accounted for (Mommer et al., 2012). My results confirm that leaf traits were significantly different among shade treatments, while roots traits were different among fertilization treatments. Therefore, these different environmental controls drive the functioning of plants’ above and belowground organs. For example, a high light, low nutrient environment will show different correlations between leaf and root traits than a high light, high nutrient environment.

### 5.3 Intraspecific trait variation

Physiological \((A_{\text{sat}}, A_{\text{mass}} \text{ and } R_{\text{mass}})\) and morphological \(\text{(LA)}\) leaf traits, as well as root traits \((\text{SRL and D})\) had the highest degree of intraspecific variation (Table 7) which is consistent with previous studies (Gagliardi et al., 2015; Pérez-Harguindeguy et al., 2013; Tobner et al., 2013; Valladares et al., 2000). Albert et al., (2010) found that while trait values are highly variable within-species, this variations and trait expression is largely dependent on environmental conditions.

I hypothesized that variation in leaf traits would be greater under shade trees. Consistent with this expectation, my results show that low light (full shade treatments) promoted more intraspecific variation in physiological traits \((A_{\text{mass}}, A_{\text{sat}}, R_{\text{mass}})\) (Table 8). Alternatively, high light conditions (monoculture and managed shade) resulted in lower levels of intraspecific variation as compared to the variation across the entire dataset in all leaf traits (Table 8). Shade trees create a
heterogeneous light environment (Beer et al., 1998; Charbonnier et al., 2013) and plants that have a wider variety of light capturing traits are more functionally diverse, which can in turn lead to greater resource partitioning (Wood et al., 2015). Alternatively, in monocultures, coffee plants show less functional trait variation (Table 8). Since the light environment is relatively homogeneous, it promotes the proliferation of a more narrow range of traits that can take advantage of high-light environments. However, there was one exception to this trend: leaf thickness had greater intraspecific variation in monoculture as compared to shade plots. It has been previously reported that leaves are thicker, with a lower LMA under full sun conditions for improved light capture (Pompelli et al., 2012). This greater range in leaf thickness would thus promote some leaves to conserve water and help the overall plants performance while others invest in leaf area for photosynthetic light capture.

My findings show that intraspecific root trait variation was not greatest under the lowest fertilization treatment as previously hypothesized. Instead, intraspecific root variation in the lowest fertilization treatment (50 kg N ha\(^{-1}\) yr\(^{-1}\)) fell within the modeled distribution of the entire dataset (Table 9). This suggests that low fertilization did not strongly affect intraspecific variation. Intraspecific variation in SRL and root D in the second lowest fertilization treatment, (110 kg N ha\(^{-1}\) yr\(^{-1}\)) was greater than the variation observed across the entire dataset, while intraspecific variation of SRL and D in the highest two fertilization treatments (170 and 230 kg N ha\(^{-1}\) yr\(^{-1}\)) was lower than the distribution of data in the entire dataset (Table 9). In these high nutrient conditions, it can be assumed that there is sufficient soil N and therefore a more homogenized soil environment. Alternatively, lower nutrient conditions may be more heterogeneous in the distribution of soil nutrients. Studies have shown that in heterogeneous soil environments, roots will proliferate in high nutrient patches of soil (Hodge, 2004; Mora et al., 2013). Root foraging strategies were presumably induced in the low fertility treatments given patchy soil nutrients, resulting in a large variation in coffee SRL under such conditions.

### 5.4 Implications for management of coffee plants

In modern coffee markets there is an increase in demand for “conscious” coffee (fair trade, organic, shade-grown and bird-friendly) which translates into lower nutrient inputs and greater integration of diversity into farms (Jha et al., 2014; Ponte, 2002). The integration of biodiversity into coffee systems provides ecosystem services (Jha et al., 2014) yet comes at the cost of yield
in most cases when proper management is not maintained (Campanha et al., 2004; Hagggar et al., 2011). By using functional traits as an indicator of plant health and growth strategy, we can optimize management to improve yields without compromising biodiversity. Low nutrient conditions promoted resource acquiring root traits (high SRL, low D) while high shade treatments induced a mixture of resource acquiring morphological leaf traits (high SLA, low LMA), and resource conserving physiological leaf traits (low A). Generally, plants which display resource acquiring traits perform better in high resource environments, since they are able to invest large amounts of energy into the growth of above and belowground tissue with a high return on investment (resources are available to be used by the plant (Reich, 2014). This large investment would be potentially detrimental to plants in nutrient poor environments as they would invest greatly in the growth of resource acquiring traits and have little return on investment (little soil nutrients or light to be captured by new growth). However, nutrient poor soils tend to promote resource acquiring root traits (high SRL) (Ostonen et al., 2007; Trubat et al., 2006) and low light environments promoted resource acquiring morphological leaf traits (Campanha et al., 2004; Pompelli et al., 2012). These are both foraging strategies by the plant whereby they invest large amounts of energy into the production of root and leaves, which will enhance nutrient and light capture. I found that low LMA did not translate into higher A (Table 5), which means that these plots are not getting a good return on investment. It is known that within species differences in leaf traits partially explain differences in coffee yield (Gagliardi et al., 2015). Therefore, looking into the relationships between functional trait expression and yield can provide important information into crop functional biology.

In addition, since agricultural systems are heterogeneous in their soil and light environments, it becomes advantageous for plants to display a larger range of resource capturing traits. Theoretically, management systems which promote greater intraspecific variation in leaf and root traits should lead to greater resource partitioning and therefore, use resources more efficiently (Wood et al., 2015). Based on this study, a high nutrient, high sun management system, similar to intensive monocultures, would promote low intraspecific variation and resource conserving root traits, while moderate fertilization (110 kg N ha\(^{-1}\) yr\(^{-1}\)) and high shade, similar to the conditions of an agroforestry system, would promote greater intraspecific variation in plant leaf and root traits, as well as resource acquiring root traits and mixture of resource acquiring and resource conserving leaf traits.
For coffee plants in similar climatic conditions as this study, the 110 kg N ha\(^{-1}\) yr\(^{-1}\) fertilization treatment, paired with a shade tree would promote the most resource acquiring leaf and root traits, and therefore promote fast-growing short-lived coffee plants. Similarly, low fertilization, high shade treatments also increase intraspecific trait variation. This increased variation in plant functional traits could lead to better resource partitioning and resource use within heterogeneous soil and light environments, which could enhance plant performance in sustainable agroecological systems.
Chapter 6
Conclusion

6.1 Conclusions

Using a functional trait approach is a novel approach to studying agroecosystems and their response to management techniques. Specifically, this study addressed how intraspecific functional traits of coffee leaves and roots change along a soil fertility and light gradient in agroforestry systems.

In line with previous studies, I report that suits of functional leaf traits fall along a resource spectrum similar to those proposed by the LES (Wright et al., 2004). We also had similar covariations between root traits as have been reported by previous studies, where high SRL corresponded to low D (Tobner et al., 2013). Coffee leaf traits varied systematically across shade treatments, and coffee root traits vary systematically across nutrient treatments. High levels of shade provided by an unpruned Erythrina trees, promoted a combination of resource acquiring (high SLA, low LMA, high SRL) and resource conserving (low $A_{mass}$) functional traits. High light levels found in monoculture plots promoted the expression of resource acquiring physiological leaf traits (high $A_{mass}$) and resource conserving morphological traits (high LMA, low SLA, low SRL). Soil fertility also had a significant effect on the expression of functional traits, although these effects were clearer for root traits rather than leaf trait expression. High fertilization (240 kg N ha$^{-1}$yr$^{-1}$) promoted resource conserving root traits (low SRL), while low fertilization (50 kg N ha$^{-1}$yr$^{-1}$) promoted resource acquiring root traits (high SRL). Fertilization did not systematically affect leaf trait expression, as the 110 kg N ha$^{-1}$yr$^{-1}$ treatment showed the highest SLA and LNC and the 170 kg N ha$^{-1}$yr$^{-1}$ the lowest SLA and LNC. The highest amounts of intraspecific variation occurred in our physiological leaf traits ($A_{mass}$, $A_{sat}$) and root traits (SRL, D) as well as LA. This falls in line with previous studies on intraspecific variation (Gagliardi et al., 2015; Tobner et al., 2013; Valladares et al., 2006). When our data was partitioned into shade and nutrient categories, increased amounts of shade (ie: the full shade condition) increased intraspecific variation in our physiological leaf traits. We also saw that a decrease in shade (ie. our monoculture plots) resulted in lower levels of intraspecific variation as compared to the variation across the entire dataset in all leaf traits for all leaf-level traits. Fertilization also influenced intraspecific variation in roots. Under high fertilization (170 and 240
kg N ha\(^{-1}\) yr\(^{-1}\)), SRL and D had lower levels of intraspecific variation as compared to the variation across the entire dataset. The lowest nutrient treatment (50 kg N ha\(^{-1}\) yr\(^{-1}\)) did not result in an increase in variation for root traits as hypothesized. Instead intraspecific variation fell within the modeled distribution of the entire dataset, meaning that this treatment did not have a significant effect on variability. The 110 kg N ha\(^{-1}\) yr\(^{-1}\) treatment was the one that showed an increase in intraspecific variation for root traits. Fertilization also affected the variability of leaf traits, with the 170 kg N ha\(^{-1}\) yr\(^{-1}\) treatment significantly decreasing variation in SLA and LMA.

Therefore, increased amounts of shade seemed to promote a combination of resource acquiring (high SLA, low LMA, high SRL) and resource conserving (low \(A_{\text{mass}}\)) functional traits, while promoting high levels of intraspecific variation in physiological leaf traits. Conversely, high levels of light prompted resource acquiring physiological leaf traits (high \(A_{\text{mass}}\)) and resource conserving (high LMA, low SLA, low SRL) morphological traits, while overall decreasing intraspecific leaf trait variation. High amounts of N-based fertilizer lead to the promotion of resource conserving root traits (low SRL, high D) and resulted in a decrease in intraspecific root trait variation. Therefore, agroforestry systems, paired with moderate amounts of fertilization would promote fast-growing, short-lived species, which display resource acquiring root and leaf traits. Proper pruning of trees to ensure an adequate amount of photosynthetically active light (ie: less than 55% shade), could shift resource conserving physiological leaf traits to the resource acquiring side of the spectrum. This management system would also see plants with greater leaf and root trait variation. This greater amount of variation in functional traits would lead to better resource partitioning, and therefore resource use efficiency, which could reduce the amount of nutrient inputs necessary for the growth of crops.

6.2 Areas of future research

Using a trait based approach to assess agricultural systems can provide information about ecosystem functioning. More research on covariation of functional leaf and root traits within species, over nutrient and light gradients could provide additional insight into the driving forces behind plant trait expression. In particular, studies on root traits are needed that account for the influence of sampling method, root diameter class and soil/fertilizer type on the proliferation and expression of traits. In combination with what we know about how functional traits change over nutrient and light gradients, management techniques could be selected for which provide
multiple ecosystem services. In addition, species-specific trait information and information on functional trait responses to environmental stimuli could be used along with modelling approaches to derive management techniques that address specific management targets.
References


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