Intraspecific trait plasticity in coffee agroforestry systems of Costa Rica

by

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Abstract

Although a common plant response to environmental gradients, leaf trait plasticity is often uncharted in agroforestry systems. The objective of this study was to examine the effect of a i) local-scale gradient (light, nutrients) induced by shade tree diversity and ii) large-scale gradient (climato-edaphic) induced by altitude on coffee plant response on multiple agroforestry research farms in Costa Rica. Results show large variability of coffee traits: leaf photosynthetic rates, specific leaf area (SLA) and number of fruiting nodes deviate along both gradients. Mean SLA increased with increasing shade tree diversity. However, with increasing altitude, full sun coffee photosynthesized at higher rates than shaded coffee. Concurrently, other coffee leaf physiological and morphological traits differentiated between full sun and shaded coffee with increasing altitude. Results suggest soil moisture and light availability dominate environmental correlates to intraspecific coffee trait plasticity, providing insight to sources of coffee performance variability in monoculture and agroforestry systems.

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Chapter 1
Introduction

1.1   Research context

Coffea arabica, herein referred to as coffee, is the world's second-most traded commodity after oil (Davis et al, 2012) and one of the most important crops for export in Central America (van Oijen et al, 2010a), employing 20 to 25 million people in this part of the world alone (Aguilar and Klocker, 2002). As of 2000, there were over 73,000 coffee producers in Costa Rica with an average farm size of 1.6 ha, representing 28% of rural labour in Costa Rica in 2002 (Varangis et al, 2003). Although this industry is an important component of the Costa Rican economy, the global coffee market is not stable, threatening the livelihoods of many farmers and motivating smallholder farmers to search for alternative coffee growing techniques (e.g. Beer et al, 1998; Haggar et al, 2011).

Coffee is a shade-tolerant perennial species (DaMatta, 2004) that can be grown in association with a variety of shade trees or in full sun conditions. Since the full sun systems do not benefit from the rich soil nutrients associated with shade tree litter fall or fixed nitrogen (N) from leguminous trees (Nygren et al, 2012b; Munroe and Isaac, 2013), nutrients must be replaced with fertilizer inputs to produce high yields (Haggar et al, 2011). These costs put further strain on coffee producers, in addition to the coffee market instability. These factors have begun to push small-scale farmers back into traditional shade-grown coffee systems (Siman, 1992; Perfecto et al, 2005). In addition to the financial impact of full sun coffee, there are many negative environmental impacts, including concerns regarding the loss of biodiversity due to the reduction or complete elimination of shade trees in coffee systems (Perfecto et al, 1996), and the contamination of watersheds due to the excessive fertilizer inputs and potential soil erosion (Reynolds, 1991; Fernandez and Muschler, 1999; Harmand et al, 2007; Haggar et al, 2011)

The apparent benefits associated with shade-grown coffee may be attributed in part to the greater heterogeneity of the plant community, which can lead to complementary plant interactions and resiliency to abiotic changes. It has been observed in natural plant communities across landscapes that positive (facilitative), negative (competitive) or complementary (neutral) interspecific plant interactions occur (e.g. Brooker et al, 2008). In coffee agroforestry literature

coffee response to shade tree presence via aboveground interactions (e.g. Campanha et al, 2004; Vaast et al, 2008) and belowground interactions (e.g. Mora and Beer, 2013; Munroe and Isaac, 2013; Nygren et al, 2012b) has been analysed. These intraspecific coffee responses have shown some trait variability to these interactions (e.g. Lambers et al, 2008; Vaast et al, 2008; Matos et al, 2009), but the complexity often limits farmers' ability to fully exploit the potential benefits of shade-grown coffee.

There is an emerging interest in intraspecific variability of plant response to environmental conditions across abiotic gradients in the ecology literature (e.g. Niinemets, 2007; Brousseau et al, 2013; Freschet et al, 2013; Isaac and Anglaaere, 2013), providing a new approach to comprehensively understand how the same plant responds to a range of conditions. Predominately, these plant responses can be categorized as resource acquiring or resource conserving traits, the direction of which is highly dependent on environmental conditions. However, this approach to understanding target plant response, coffee in this case, is limited in the agroforestry literature. Despite the well-known variability in plant traits in natural plant communities and preliminary findings of variability in coffee plant traits (e.g. Chaves et al, 2008; Cavatte et al, 2012; Matos et al, 2013), most coffee agroforestry research still focuses on single plant response to treatment effects. Little work has examined the coffee intraspecific plasticity in farmer's fields related to shade tree presence across environmental gradients. Furthermore, studies are often conducted in a single area, which becomes problematic when attempting to extrapolate the data to other sites, especially with the use of simple dynamic models. These models are used to predict coffee yield outcomes given certain parameters, including abiotic factors and plant physiology (van Oijen et al, 2010a). In order to overcome this current limitation, it has been suggested that multi-factorial studies be conducted across sites (e.g. Beer et al, 1998; van Oijen et al, 2010a). It is important to understand the plasticity of coffee plant traits across abiotic gradients, as coffee agroforestry systems are inherently heterogeneous environments.

1.2 Research questions and hypotheses

In order to address the current gaps in coffee agroforestry research, I analysed coffee leaf trait plasticity along a i) local scale gradient (light and nutrients) induced by shade tree diversity and ii) large scale gradient (climato-edaphic) induced by altitude. The objective of this study was to

examine the effect of such environmental gradients on coffee plant response in multiple on-farm research sites of increasing shade tree species richness and altitude in the Cartago and San José regions of Costa Rica.

According to the concepts above, I proposed the following hypotheses for the studied coffee systems:

1   Coffee leaves will exhibit trait plasticity along environmental gradients in managed coffee systems due to multiple and potentially simultaneous abiotic factors. This plasticity will manifest as alterations in resource acquiring or resource conserving traits of leaves.

2   At the local scale, shade tree presence will not inhibit coffee performance (leaf physiology, leaf morphology, plant productivity). There will be a multitude of interspecific interactions driving intraspecific leaf trait variations.

3   At the landscape scale, lower altitudes along a climato-edaphic gradient will inhibit coffee performance (leaf physiology, leaf morphology, plant productivity). However, this inhibition will be moderated by shade tree presence.

1.3   Research significance

The recent shift back to shade-grown coffee is mirrored by the popularity of this coffee product in Western culture. Its growing popularity may be attributed to large coffee shops specializing in shade-grown coffee, or it may be due to greater consciousness of environmental degradation. Regardless of the reason for its popularity, the design and process of coffee agroforestry systems need to be analysed and readjusted in order to achieve optimal yield stabilities. Research thus far has focused on testing different agroforestry management techniques in order to determine optimal design. There are many components to be considered when determining management techniques, such as shade tree species selection, density, pruning, type and timing of inputs, and level of biodiversity within the system. These management decisions affect target species (coffee) plant response, which in turn affects overall yield. Exploring coffee response to such management variables with a trait-based approach across gradients will provide insight into optimal design under a range of conditions.

Chapter 2
Coffee Agroforestry

2.1 History of coffee agroforestry

Coffee is a perennial shrub that originated in the forest understory of the Ethiopian highlands (Kumar, 1979; Berthaud and Charrier, 1988). Due to its evolutionary background as an understory shrub, coffee is often considered a shade-tolerant plant species and early plantations of coffee mimicked shade environments with coffee planted under overstory trees (DaMatta, 2004). However, it has been observed that coffee plant yields are highly plastic in different light conditions, and can produce greater cherry yields when grown in full sun (e.g. Beer et al, 1998; Haggar et al, 2011). Due to the potential to produce more coffee, the practice of shading was abandoned during the last century for full sun monoculture systems in many coffee producing regions. The switch in Brazil was almost complete by the 1950s (DaMatta, 2004), and by 1990, half of the area of coffee plantations in Latin America was estimated to be intensive monoculture systems with one shade tree species or none at all (Perfecto et al, 1996).

The ability of coffee plants to acclimate to the drier climate and greater irradiance in monoculture systems is believed to stem from the thousand years coffee cultivars grew in drier climate without shade before being introduced to Asia and Latin America (van der Vossen, 2005). Furthermore, many coffee cultivars planted today have been improved in full sun conditions (DaMatta and Rena, 2002), often leading to enhanced productivity compared to shaded coffee. However, full sun coffee requires a larger amount of fertilizer inputs compared to shaded coffee systems (e.g. Haggar et al, 2011). The greater amount of inputs has led to regional environmental problems, including nutrient leaching, soil erosion, and water contamination (Harmand et al, 2007). These negative environmental effects, combined with the high cost of artificial inputs, have motivated a return to shaded coffee systems by smallholder coffee farmers and a resurgence in research highlighting the many benefits in coffee agroforestry systems (e.g. Harmand et al, 2007; Haggar et al, 2011).

Recent research has found that growing coffee beneath shade trees can delay ripening, which increases coffee cherry size and improves cherry chemical composition for superior beverage quality compared to sun-grown coffee (Muschler, 2001; Vaast, 2006; Vaast et al, 2008). Shade

trees can also create an alternative source of income for farmers through the cultivation of their fruit or timber (DaMatta et al, 2007). Coffee agroforestry provides many ecosystem services, such as enhanced soil fertility and reduced soil erosion (Babbar and Zak, 1994), as well as improved water quality (Somarriba et al, 2004) compared to unshaded coffee. However, coffee agroforestry is not often recommended for optimal or near-optimal climato-edaphic conditions, as shading is not very beneficial and can be detrimental to certain coffee plant processes (DaMatta et al, 2007). Conversely, coffee agroforestry is most recommended in suboptimal coffee growing locations with harsh climate conditions, because shade trees are able to moderate, and thus improve, microclimate conditions beneath their canopies. Shade tree presence in coffee systems enhances microclimate conditions through the reduction of wind speeds, regulation of temperature, increase in relative humidity, lower radiation input and, if properly managed, an increase in water-use efficiency (DaMatta et al, 2007), contributing to better coffee plant health and more stable coffee production in agroforestry systems (Beer et al, 1998; Soto-Pinto et al, 2000; DaMatta et al, 2007). Shade trees' ability to moderate microclimate conditions may also serve as a method to reduce the negative impacts of climate change for those without access to new technology (Lin, 2007), such as hardier cultivars.

2.2   Interspecific plant interactions in coffee agroforestry

Interspecific plant interactions in agroforestry systems are very complex, and can range from negative (competition) to positive (facilitation) effects, both above and belowground and either spatially or temporally (Vandermeer 1989; Schroth, 1999; Jose et al, 2004). According to Tilman (1999), the importance of interactions depends on the heterogeneity of resources and abiotic factors in the system. Therefore, as a system increases in heterogeneity, the role of facilitation in the system increases (Fridley, 2001). In an agroforestry system, plant interactions are greatly influenced by variations in management techniques and climato-edaphic conditions. The pathways of competitive or facilitative interactions can be categorized into i) light, ii) nutrients and iii) water. Resource partitioning may result from differences in resource types, required amounts, and resource pools. By utilizing differences in resource requirements, the resource-use efficiency of the system increases (Hooper and Vitousek, 1998).

2.2.1   Light resources

Depending on the shade tree species selected for the agroforestry system, as well as the density of the shade trees, the amount of incoming light penetrating through the shade tree canopy can vary greatly. Reduced amounts of incoming solar irradiance are due to light interception by the shade tree canopy, which may lead to reduced photosynthetic rates due to light limitation (DaMatta, 2004). Coffee grown under 80% shade cover has sharply decreased photosynthetic rates, as observed in glasshouse experiments in Campinas, Brazil (Carelli et al, 1999) and in-field experiments in Viçosa, Brazil (Matos et al, 2009). Reduced irradiance also reduces the evaporative demands of the crop leaves and soil, increasing plant water and soil moisture. Therefore, while photosynthetic rates of coffee grown in shade are limited by light interception, the photosynthetic rates of coffee grown in full sun are limited by stomatal conductance. This is because stomata close when the leaf-to-vapour air pressure deficit drops due to drier air, thus restricting the rate of incoming carbon dioxide (CO_2) (DaMatta, 2004).

In addition to an effect on photosynthetic rates, shade trees have a positive effect on coffee yield when shade cover is between 23 and 38% (Soto-Pinto et al, 2000). In Costa Rica, Haggar et al (2011) observed variability in coffee yields across treatments of full sun and under shade with *Terminalia amazonia, Erythrina poeppigiana,* and *Inga laurina*, which differ in height, crown diameter and shade cover. This variability in coffee may be due to i) changes in carbon assimilation rates of the whole plant (Beer et al, 1998; DaMatta et al, 2007), ii) balances between foliage growth and flowering (Cannell, 1976), and iii) differences in the amount of nodes formed per branch (Montoya et al, 1961; Castillo and López, 1966).

2.2.2   Nutrient resources

The increased heterogeneity of plant composition in a coffee agroforestry system has a large impact on belowground interactions, positive, negative, or neutral. Management decisions and climato-edaphic conditions greatly dictate the favourability of the interspecific interactions. I will focus on the nutrient dynamics in coffee agroforestry according to three aspects: 1) dinitrogen fixation; 2) litter decomposition; 3) nitrogen (N) mineralization.

2.2.2.1　Dinitrogen fixation

Dinitrogen (N_2)-fixing shade trees, or service trees, are able to provide agroforestry systems with more nutrients compared to other shade trees, due to their ability to fix atmospheric N through bacteria symbioses. The N that is fixed is made available to the crops via i) decomposition of organic material (e.g. litterfall, pruning material, senescing root material), ii) direct transfer through root exudation, and iii) a common mycelial network of mycorrhizae-forming fungi (e.g. Khanna, 1998; Nygren et al, 2012ab; Munroe and Isaac, 2013). This N is then available to be taken up by associated crops in agroforestry systems (Nygren and Leblanc, 2009). However, many of the facilitative effects produced by N_2-fixing shade trees decrease as the distance between the plant components increases.

N_2-fixing shade trees can also compete with coffee crops belowground during times of pruning. It has been observed in a coffee agroforestry system that when *E. poeppigiana* is pruned, there can be a decline in the amount of active N_2-fixing nodules (Nygren and Ramírez, 1995). This decline may be due to the plant's high sensitivity to the pruning process (Chesney and Nygren, 2002). Re-initiation of nodules does not begin until at least 10 weeks after pruning (Nygren and Ramírez, 1995; Chesney and Nygren, 2002). During this time of limited active N_2-fixing nodules, *E. poeppigiana* must depend on the N in the soil for its nutrient source, indicating the potential for competition with other plants in the system. To reduce the amount of competition that results, management may reduce the extent of pruning (Chesney, 2008) or alter the pruning schedule (Muñoz and Beer, 2001).

2.2.2.2　Litter decomposition

Shade tree pruning regimes are important for both the regulation of canopy cover aboveground and the contribution of organic material belowground. The pruning debris may be left on site to decompose into organic matter, which improves nutrient recycling, soil fertility and physical soil structure (García-Barrios and Ong, 2004; Somarriba and Beer, 2011). Through the process of decomposition and subsequent mineralization, the litter that remains on site eventually gets converted into nutrients for the plant community. When this management technique is practiced, there is a greater concentration of available nutrients (e.g. N, phosphorus, and potassium) beneath the tree crowns (Isaac et al, 2007; Yadav et al, 2008). This decomposition process

depends on the species present and the climate region and microclimate conditions (Hossain et al, 2011). As previously mentioned, the litter from N_2-fixing shade tree species is of higher quality (Kelty, 2000), which improves nutrient cycling around the shade trees (Isaac et al, 2007), promoting nutrient transfer into the system for plant uptake.

2.2.2.3   Nitrogen mineralization rates

Decomposing organic matter must undergo mineralization processes to convert the organic N within the plant litter into inorganic N via microbial action, in order to be available for uptake by the plant community. N mineralization can be separated into three processes: ammonification, nitrification and immobilization. The breakdown of organic N to inorganic N is described by the processes of ammonification and nitrification. However, when microbes take up inorganic N for their own use, due to an insufficient supply of their principle energy source, organic N, immobilization occurs, thereby depleting the resource pool for the plant community (Chapin et al, 2002).

When comparing coffee agroforestry to unshaded systems, studies have observed higher rates of N mineralization when shade is incorporated. For example, coffee systems with *E. poeppigiana* have higher available soil N (Babbar and Zak, 1994) and higher rates of N mineralization (Hergoual'ch et al, 2007) compared to unshaded coffee. However, not all studies have confirmed this trend. For example, in a coffee system with *Eucalyptus deglupta* shade trees and a monoculture system, no difference in N mineralization rates was observed (Harmand et al, 2007). This inconsistency is unexpected, as shade trees improve the microclimate beneath the canopy (Nygren and Leblanc, 2009), such as regulating extreme temperatures (Lott et al, 2009), thus improving mineralization rates. A potential cause for this discrepancy is the difference in the shade tree species selection. Greater mineralization rates are often observed in systems containing leguminous trees, which may improve litter quality, thereby improving mineralization rates (Nygren and Leblanc, 2009). Furthermore, differences in climato-edaphic conditions may result in differences in mineralization rates. The temporal variation of rainfall patterns leads to variations in the necessary phases of the mineralization process, such as the dissolution of organic N and the diffusion of ammonium ions to the nitrifying bacteria (Olsen and Kemper, 1968; Stark and Firestone, 1995). Babbar and Zak (1994) found that in a coffee agroforestry system in the Central Valley of Costa Rica, net N mineralization and net nitrification rates were

lowest during the driest months and highest during the wettest months. This relationship between mineralization rates and moisture is similar to that of the decomposition process, where regions that experience dry seasons may consequently experience lower soil N.

2.2.3   Soil water resources

In addition to aboveground canopy architecture and soil nutrient contribution, shade tree species selection should also focus on rooting architectures. When two or more species have similar rooting systems, either vertically or laterally, belowground competition is more likely, since the plant roots access the same area for soil resources (van Noordwijk et al, 1996). When this occurs in agroforestry, it often results in the reduced performance of one of the component species (Jose et al, 2000; Nygren et al, 2012a). However, if species of different rooting architectures are paired, complementarity for soil resources may occur. It is known that coffee is a shallow-rooted crop, having roots within the first 0.3 m of the soil surface to a distance of approximately 0.75 m from the stem (Sáiz del Rio, 1961; Inforzato and Reis, 1973; Huxley, 1974; Alfonsi, 2005). Furthermore, coffee roots can be very plastic due to variations in plant density (Cassidy and Kumar, 1984; Rena et al, 1998), soil characteristics (Rena and DaMatta, 2002), competition from weeds (Ronchi, 2007) and ontogeny (Inforzato and Reis, 1973; Bragança, 2005). When coffee is paired with shade trees that have a greater proportion of fine roots in deeper soil layers, such as *Terminalia* (Garcés, 2011), the two plant species access different resource pools on a spatial scale, and so do not interfere with each other. The shade tree, in this situation, acts as a "safety net" for the system (van Noordwijk et al, 1996; Garcés, 2011), as the shade trees' roots take up soil water that have been leached out of the topsoil to deeper soil strata, thus increasing the resource-use efficiency of the system.

2.3   Plant trait plasticity in coffee agroforestry

Plant traits can strongly vary based on differences in resource allocation. Plant functional traits have been defined as any plant feature that has a potentially significant impact on establishment, survival, and health, and can be related to resource-conserving and resource-acquiring traits (Reich et al, 2003). Therefore, when plant functional traits vary within a species in response to natural or management-induced environmental conditions, the plant is altering between conserving and acquiring either above or belowground resources, such as those described above.

This intraspecific plasticity in functional traits has been observed in natural plant communities (e.g. Freschet et al, 2013; Hajek et al, 2013) and managed agroforestry systems (e.g. Chaves et al, 2008; Cavatte et al, 2012; Isaac et al, 2014).

Specifically for leaves, the worldwide leaf economic spectrum (LES) quantifies different species along a spectrum of investments in resources from quick returns to slower potential returns (Wright et al, 2004). However, it has been observed that environmental variables can affect these patterns of resource allocation (Liu et al, 2010; Poorter et al, 2012). As a result, intraspecific variation in plant traits is manifested. In natural plant communities, both above and belowground interspecific and intraspecific interactions combine with inherent heterogeneous environmental conditions to promote intraspecific plant trait plasticity. For example, Brousseau et al (2013) observed that morphological and physiological traits demonstrated different optima according to small spatial scale environmental variations in two tree species (*Eperua falcate* and *E. grandiflora*, Fabaceae) in a tropical rain forest. Furthermore, it was concluded that the ecological processes that stimulate interspecific variations in plant traits also act on intraspecific variations.

Due to observations in plant trait plasticity in natural plant communities, Valladares et al (2000) developed the phenotypic plasticity index as a method of plasticity quantification. This proposed method uses maximum and minimum mean values for selected plant traits to create a plasticity index value between 0 and 1, where 0 is limited plasticity and 1 is high plasticity. This index has been used in natural plant communities, for example with rainforest shrubs (*Psychotria*, Rubiaceae) in Panama (Valladares et al, 2000), holly (*Ilex aquifolium* L.) in Mediterranean fields of central Spain (Valladares et al, 2005), and an understory evergreen shrub (*Rhododendron ponticum*) in southern Spain and Belgium (Niinemets et al, 2003). Although limited, the plasticity index has also been used for coffee to determine changes in coffee trait plasticity over a temporal gradient (Chaves et al, 2008), across different light treatments (Matos et al, 2009), and in different light and water treatments combined (Cavatte et al, 2012).

2.3.1 Plasticity in coffee leaf and reproductive traits

Coffee leaf size is often cited in the literature as a highly plastic coffee plant trait, likely because it is often easily measured. It has been observed that shaded coffee has leaves that are 25% larger compared to full sun coffee, with a shoot-to-root ratio 53% larger (Lambers et al, 2008).

Furthermore, coffee leaf thickness is often greater in full sun coffee leaves, due to i) larger palisade mesophyll, ii) denser stomata (Matos et al, 2009), and iii) higher concentration of lipids (Lambers et al, 2008).

This variability in leaf morphological traits has direct effects on the variability of photosynthetic rates, since coffee leaf-level photosynthesis is limited by either the photosynthetic photon flux density or the concentration of CO_2 in the mesophyll layer of the leaf (Farquhar et al, 1980), which in turn is primarily controlled by stomatal conductance (G_s) (Franck and Vaast, 2009). Research has found a large amount of variability in coffee leaf level photosynthetic rates, as it can range between 4-11 µmol m^{-2} s^{-1}, given current natural atmospheric CO_2 concentrations and saturating light (Ceulemans and Saugier, 1993; Silva et al, 2004; Franck et al, 2006). Within a single coffee plant, sun leaves have higher rates of dark respiration and photosynthesis with less susceptibility to photoinhibitory stress compared to coffee shade leaves (e.g. Ramalho et al, 2000; Walters, 2005; Niinemets, 2007; Vaast et al, 2008). Furthermore, coffee leaf N requirements vary between sun and shade leaves (Walters, 2005; Niinemets, 2007), highlighting the variability in the acclimation of leaves in different positions within the coffee canopy.

An increase in coffee yield in full sun conditions has been associated with increased photosynthetic rates (DaMatta, 2004), growth trade-offs (Cannell, 1976; DaMatta et al, 2007), and node production (Montoya et al, 1961; Castillo and López, 1966; Cannell, 1976). However, these results are dependent on site-specific conditions, as there have also been observations of higher yields in shaded systems (Vaast et al, 2008). This observation may be further complicated by the species of shade trees present. It has been observed that coffee grown beneath *Terminalia* experienced lower production compared to coffee grown beneath *Eucalyptus*, while coffee grown beneath *Erythrina* produced similar yields to full sun coffee (Vaast et al, 2008). Trade-offs between vegetative growth and yield may be due to differences in timing, potentially influenced by a difference in climatic preferences (Maestri and Barros, 1977; Barros et al, 1999), or competition between the two processes for available resources (DaMatta et al, 2007). In addition to final yield measurements, coffee beverage quality varies when grown in different environmental conditions. For example, when grown under reduced light exposure, cherry ripening is delayed, which may contribute to increased caffeine and fat content compared to full sun coffee (Vaast et al, 2008).

Although data is limited, some research shows trait plasticity across climato-edaphic gradients. The optimal coffee growing regions are at altitudes of 1600-2800 m.a.s.l. (DaMatta et al, 2007), though coffee can be, and frequently is, grown at altitudes beyond this range. This altitudinal range is optimal for coffee growth due to its climatic characteristics (DaMatta, 2004). The optimum mean annual temperature range for coffee is 18-21°C (Alègre, 1959). At annual temperatures below 18°C and above 25°C, coffee plant response changes, as reduced vegetative growth (DaMatta et al, 2007), reduced yield and altered chemical composition of coffee cherries (Franco, 1958; Camargo, 1985) have been reported. Similarly, the optimum annual rainfall is 1200-1800 mm (Alègre, 1959), although this range is highly dependent on specific soil characteristics, humidity and cloud cover, as well as management regime (DaMatta et al, 2007). Coffee requires a short dry spell of 2-4 months in order to stimulate flowering (Haarer, 1958). Without this dry period, coffee plants respond with lower and temporally scattered yields (DaMatta et al, 2007).

2.4 Gaps in the literature

Intraspecific trait plasticity research to date has predominately focused on natural plant communities in the ecology literature. This trait-based approach is limited in coffee agroforestry literature, due to the common approach to study managed systems, where research looks at single plant response to confined experimental or management treatment effects. In doing so, climatic and edaphic variables are often not considered, and so the research becomes site-specific and more difficult to be extrapolated to other sites. In order to overcome this obstacle, models can be made, due to the assumption that the underlying mechanisms of coffee plant response are not site-specific (van Oijen et al, 2010a). However, to create an effective model, it has been suggested that multi-factorial studies be conducted across sites (e.g. Beer et al, 1998; van Oijen et al, 2010ab), thereby capturing a range of plant responses across biotic and abiotic gradients, that are both controlled (through management decisions) and natural (through site conditions) that can more easily be applied to naturally heterogeneous on-farm agroforestry systems.

Chapter 3
Site Description and Methodology

3.1 Site descriptions

Three coffee agroforestry research sites were selected for this study (see Figure 2). These sites were selected in order to represent the range of environmental conditions in which coffee plants grow, as the selected sites vary in altitude (climatic differences) and management practices, including pruning schedules and fertilization rates.

3.1.1 Centro Agronómico Tropical de Investigación y Enseñanza Research Plot

Coffee plants from the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), located in Turrialba, Costa Rica were sampled. The site is located at 09°53′44" North and 83°40′7" West, at 685 m.a.s.l, with 3200 mm of annual rainfall and no marked dry season (Haggar et al, 2011) and a slope of less than 1% (Mora and Beer, 2013). Based on data from 1998-2004 collected from the on-site meteorological station, the mean temperature is 23.4^{o}C, vapour pressure 2.36 kPa, global radiation 15 MJ m^{-2} d^{-1} and wind speed 0.75 m s^{-1} (van Oijen et al, 2010a). Soils are classified as Typic Endoaquepts and Typic Endoaquults and characterized as mixed alluvial with a poor or medium fertility (Mora and Beer, 2013). Prior to 2000, the site was used as a commercial sugar cane farm (*Saccharum officinarum*) (Mora and Beer, 2013). Currently on the CATIE research plot treatments consist of coffee monoculture with no shade and coffee with shade trees in additive combinations: *Erythrina poeppigiana*, *Terminalia amazonia,* and *Chloroleucon eurycyclum*. There are also four sub-treatments based on nutrient inputs and pest management, which include intensive conventional, medium conventional, intensive organic and low organic. Each of these treatments is repeated in 3 blocks. For the purpose of this study, only the medium conventional treatment was utilized with an application of 150 kg nitrogen (N) ha^{-1} year^{-1}, which represents the standard practice of local farmers, and sampling occurred in each of the 3 blocks to maximize potential spatial variability.

In August and October 2000, coffee (*Coffea arabica* L. var. Caturra) was planted at a spacing of 1m between crops and 2m between crop rows (with a density of 5000 plants ha^{-1}) and shade trees

(*E. poeppigiana, T. Amazonia, C. eurycyclum*) were planted at a spacing of 4 m between trees and 6 m between tree rows. Replanting occurred in November 2000, due to coffee mortality as a result of initial drainage issues, which was rectified through the establishment of deep drainage channels. At the time of sampling (May 2013), shade trees had a spacing of 12 m between trees and 8 m between tree rows (108 trees ha^{-1}). In plots with exclusively *E. poeppigiana*, there was an additional severely coppiced *E. poeppigiana* situated within the middle of this 12x8 m rectangular pattern (185 trees ha^{-1}). These alterations occurred in 2007 as a shade management technique (Munroe, 2013).

After each harvest, coffee plants are selectively stump pruned based on the productive potential of each plant. In treatments containing *E. poeppigiana*, there are two annual prunings where the main trunk is pruned to a height of 1.8-2.0 m (pollarding) and the pruned material is left on site, again representing standard practices by Costa Rican farmers (Mora and Beer, 2013). The lower branches of *T. amazonia* and *C. eurycyclum* are pruned annually and the pruned material is left on the ground (Haggar et al, 2011; Campbell, 2012).

3.1.2   Aquiares Research Plot

The Aquiares research farm is located in the Reventazón river basin on the Turrialba volcano. Within the 6.6 km^2 farm, the study site was selected adjacent to the meteorological tower located at 09°56'19" North and 83°43'46" West, with an elevation of 1020 m.a.s.l. and no significant slope. Based on data from 1973-2009, this study site has a mean annual rainfall of 3014 mm and, similar to CATIE, lacks a dry season. The mean monthly air temperature ranged from 17.0-20.8^{o}C in 2009, net radiation between 5.7-13.0 MJ m^{-2} d^{-1} and wind speed from 0.4-1.6 m s^{-1} (Gómez-Delgado et al, 2010). Soils are classified as andisols according to the USDA soil taxonomy. Soils are conventionally managed with high fertilizer inputs (258 kg N ha^{-1} year^{-1}) (Charbonnier et al, 2013).

The Aquiares research farm is planted with coffee (*Coffea arabica* L., var Caturra) with *E. poeppigiana* shade trees that are not pruned. The coffee bushes have a current age of over 30 years with a planting density of 6300 plants ha^{-1}. The *E. poeppigiana* shade trees were planted at a density of 12.8 tree ha^{-1} and currently have a canopy height of approximately 20 m (Gómez-Delgado et al, 2010). There is one selective stump pruning of coffee bushes each year. The

management of the *E. poeppigiana* trees does not involve annual pruning, and so the canopy cover is approximately 12.3% (Gómez-Delgado et al, 2010).

3.1.3   Llano Bonito Research Plot

The Llano Bonito watershed is located within the central mountains of the Tarrazú/Los Santos region of Costa Rica. Within the Llano Bonito watershed, one farm was selected for sampling. This farm is located within the district of 09°40'03" North and 84°06'32" West, at 1500 m.a.s.l. and a mean slope of 35° oriented southeast. Based on data collected between 2010 and 2011 from the on-site meteorological station, this study site has a mean wind speed of 0.44-0.07 m s^{-1}, temperature between 18.2-18.7^{o}C, relative humidity between 85.3-86.5% and rainfall 2000-3830 mm (Meylan, 2012). Soils are classified as ultisols. Soil nutrient application ranged from 424-449 kg N ha^{-1} between 2010 and 2011.

The studied farm in Llano Bonito is planted with coffee (*Coffea arabica* L., var Caturra) grown under full sun, *E. poeppigiana,* or banana and plantain tree varieties (*Musa* spp). The coffee bushes are planted at a density of 6860 plants ha^{-1} with shade trees planted at a density of approximately 480 trees ha^{-1} (field surveys, Bhattarai, 2013). Annually, about 15% of coffee plant material is pruned and *E. poeppigiana* is pollarded (field surveys, Meylan, 2009).

3.2   Sampling design

Two sampling designs with similar protocols were undertaken to address my research hypotheses. I operationalized shade tree species presence to achieve a biodiversity gradient and research plot location with and without shade to achieve a climato-edaphic gradient. Coffee sampling was consistent across all imposed treatments.

The biodiversity gradient consisted of four treatments. Given the establishment of a variety of shade trees at the CATIE research plot, this portion of the study was conducted exclusively at this site. Shade tree diversity treatments are: full sun (FS), *E. poeppigiana* (shade*1), *E. poeppigiana* and *T. Amazonia* (shade*2), and *E. poeppigiana*, *T. Amazonia*, and *C. eurycyclum* (shade*3) (Figure 1). Five coffee plants were selected in each treatment and repeated in 3 blocks, for a total of 15 coffee plants per treatment. In plots containing shade trees, an *E. poeppigiana*
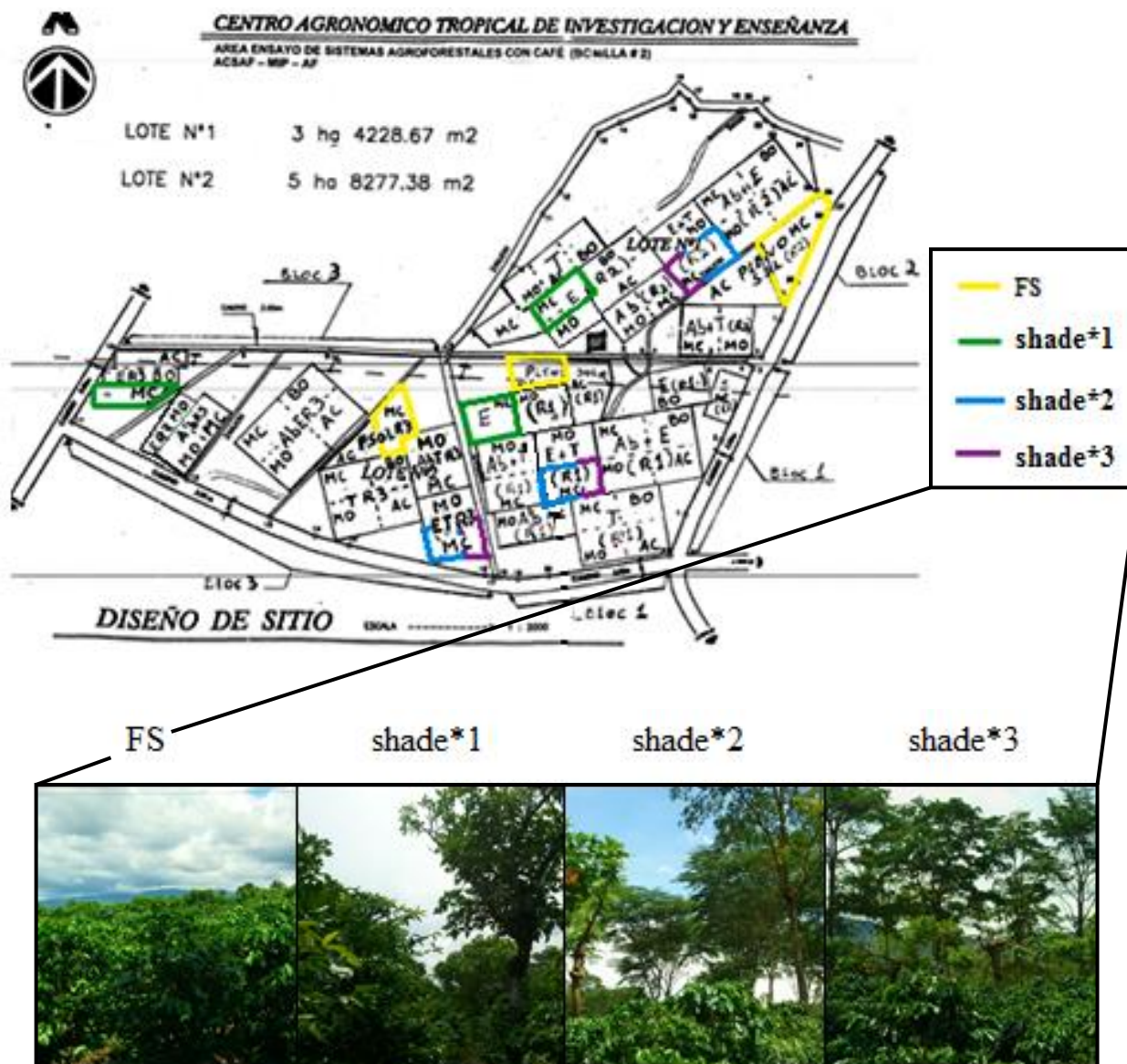
Figure 1. Map of CATIE farm with plots highlighted according to biodiversity treatments: full sun (FS), *E. poeppigiana* (shade*1), *E. poeppigiana* and *T. Amazonia* (shade*2), and *E. poeppigiana*, *T. Amazonia*, and *C. eurycyclum* (shade*3). Photographs of each biodiversity treatment are provided.

tree that was of average height and size was used as the principle tree of the plot. Coffee plants were selected within a 3 m radius surrounding the principle tree. Each coffee plant sampled was estimated to be between 3 and 4 years old since last stump pruning, and demonstrated active productivity. In FS treatments, a similar sampling technique was applied.

In order to develop a climato-edaphic gradient under which coffee plant response could be measured, different sites were selected of varying altitude, soil conditions and nutrient management regimes: CATIE (low altitude), Aquiares (mid-altitude) and Llano Bonito (high altitude) (Figure 2). At each site, two treatments were identified: full sun (FS) and shaded under exclusively *E. poeppigiana* (shade). Similar to sampling in the shade tree biodiversity treatments as described above, five coffee plants were sampled in three imposed blocks at each site and treatment (FS or shade), for a total of 15 coffee plants per treatment per site. At CATIE, all subplots were selected within Block 2 under medium conventional management. At Aquiares and Llano Bonito, subplots were selected surrounding the meteorological stations.

3.3   Shade tree measurements

3.3.1   Shade tree biomass

In the treatments containing shade trees, aboveground biomass of the shade trees was estimated using tree height and diameter at breast height (DBH) through the equation presented by Brown et al (1989) for aboveground biomass in tropical moist forests according to life zone group. Tree height was measured with a clinometer and DBH was measured with DBH tape. Since the study sites are located in tropical climate zones that lack a dry season, the equation is:

$$Y = \exp (a + b * \ln (DBH^2 * h)) \qquad\qquad (1)$$

Where:

Y = tree biomass (g)

a = calculated constant parameter (-3.1141)

b = calculated constant parameter (0.9717)

DBH = tree diameter at breast height (cm)

h = total tree height (m)
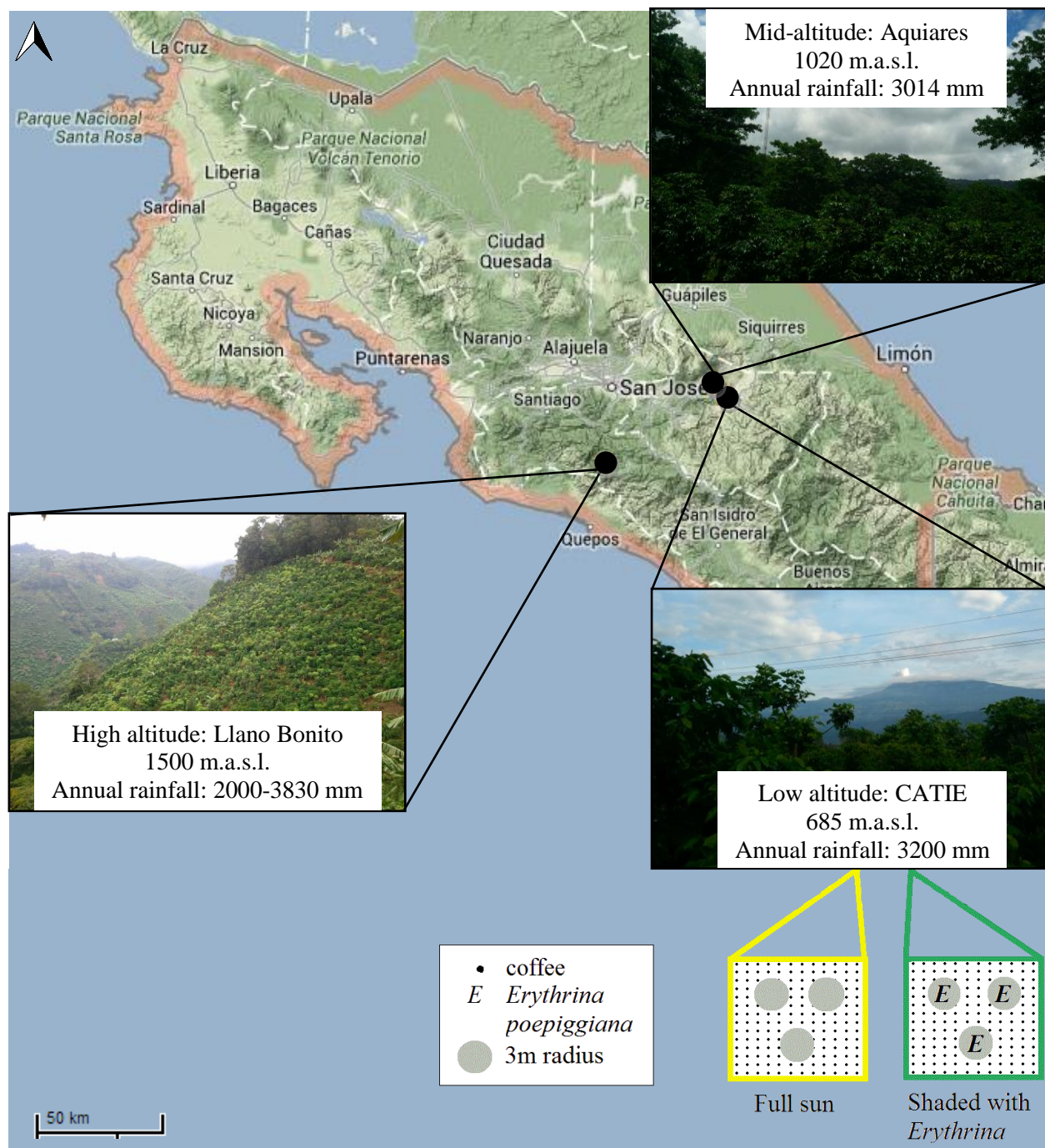
Figure 2. Map of Costa Rica with the low altitude (CATIE), mid-altitude (Aquiares) and high altitude (Llano Bonito) sites highlighted with corresponding altitude and rainfall data. Sampling designs for the full sun and shade systems are depicted. Map data © 2014 Google.

3.4 Shade level

Light levels above each sampled coffee plant were quantified using hemispherical photography between 07:00 and 10:00 on days with overcast conditions. A Nikon Coolpix 950 digital camera was used with a Nikon Fisheye Converter FC-E8 0.21x lens. Photographs were captured at a height equivalent to the sampled coffee plant height, in order to represent conditions incident on the coffee canopy, at a distance of 1.5 m from the base of the plant in the direction of the principle shade tree oriented north. Hemispherical photographs were processed and analysed with Gap Light Analyser (Simon Fraser University, 1999) for total light transmission, total diffuse light and canopy openness (n= 15 per shade treatment per site).

3.5 Coffee measurements

3.5.1 Coffee plant biomass

Coffee plant height was measured with a clinometer and trunk diameter at 15 cm above the ground was measured with diameter tape. Coffee plant diameter was not measured at breast height because the coffee plants sampled were frequently stump-pruned, a traditional management practice. Coffee plant height and diameter were then used in an equation outlined by Segura et al (2006) to estimate aboveground biomass of coffee plants growing in agroforestry systems.

$$\text{Log10 } (B_T) = a + b * \text{Log10 } (d_{15}) + c * \text{Log10 } (h) \tag{2}$$

Where:

Log10 (B_T) = log of coffee tree biomass (kg plant^{-1})

a = calculated constant parameter (-1.113)

b = calculated constant parameter (1.578)

d_{15} = diameter at 15cm height (cm)

c = calculated constant parameter (0.581)

h = coffee plant height (m)

3.5.2   Coffee leaf physiological measurements

Instantaneous leaf carbon assimilation rates and stomatal conductance using a CO_2 infrared gas analyzer (LI-COR 6400 XT, LI-COR Biosciences, Nebraska, USA) were collected for coffee leaves (n= 15 per treatment per site). Measurements were made on the third set of recently fully expanded leaves from the branch tip on branches that demonstrated productivity that were located at a height of approximately 60% of total plant height (Charbonnier et al, 2012) (Figure 3). A broadleaf chamber was used under ambient microclimate conditions with CO_2 levels held constant at 388 µmol CO_2 mol^{-1} (to reflect ambient condition) and leaf temperature and relative humidity monitored to remain within 25-27°C and 50-80%, respectively. Leaf measurements were taken between 07:00 and 09:00 to capture peak gas exchange (DaMatta et al, 2007) in May and June 2013. Light levels in the leaf chamber were set to zero µmol m^{-2} s^{-1} for dark respiration (Rd) and measurements were taken after stabilization of flux values (approximately 3-5 minutes). After Rd values were recorded, light levels were set to 1500 µmol m^{-2} s^{-1} for light saturation for photosynthesis (A_{sat}). During measurements for Rd and A_{sat}, stomatal conductance (G_s) was recorded. Saturating irradiance has been found to range between 300 to 700 µmol photons m^{-2} s^{-1}, where shade leaves have the lowest irradiance values (Kumar and Tieszen, 1980; Fahl et al, 1994). However, values below 300 µmol photons m^{-2} s^{-1} and up to 1500 µmol photons m^{-2} s^{-1} have also been found in the literature (e.g. Rhizopoulou and Nunes, 1981; Friend, 1984; Nunes, 1988; Campbell, 2012). This variability in coffee leaf-level saturating irradiance may be due to technological limitations in imitating natural conditions, the high variability between leaves with different positions in the plant canopy, among others. For these reasons, it has been proposed that coffee canopy saturating irradiance is much higher than 600-700 µmol photons m^{-2} s^{-1} (DaMatta, 2004). Therefore, an irradiance of 1500 µmol photons m^{-2} s^{-1} was used to ensure a saturating irradiance level. A_{sat} values were used with specific leaf mass (as described below) to determine mass-based photosynthesis (A_{mass}).

3.5.3   Coffee leaf morphology and nutrients

Each leaf that was sampled for instantaneous leaf carbon assimilation rates was collected after measurement. Leaf thickness was measured with electronic calipers from the tip into the centre of the leaf adjacent to the midrib. Leaf samples were traced and scanned for analysis with ImageJ 1.45 software (Wayne Rasband, National Institutes of Health, USA) to determine leaf size. Wet

Figure 3. Standard sampling protocol for coffee leaves from the third pair of opposite leaves from a productive branch tip at 60% total plant height.

weights of each leaf were recorded and then dried at 65°C for at least 72 hours, after which leaf dry mass was immediately recorded. Using leaf size and leaf dry mass, specific leaf area (SLA= leaf area/oven dry mass) and specific leaf mass (SLM= 1/SLA) were calculated. Leaf dry matter content (LDMC), sometimes referred to tissue density, was calculated using leaf wet and dry weights (LDMC= oven dry mass/wet weight).

After drying, leaf samples were ground into a powder using a Retch ball mill. Approximately 2 mg of sample was weighed on a microbalance and placed in sealed tin capsules. Total leaf N and total leaf carbon (C) were measured with a CHN analyzer (Thermo Flash 2000, Thermo Scientific, MA, USA). Calibrations with aspartic acid were performed prior to each sample run. Aspartic acid was also tested throughout analyses to ensure accuracy. Total leaf N was used to calculate leaf N concentration (LNC) and leaf N content (LNContent), which also incorporated leaf size. Photosynthetic N use efficiency (PNUE) was calculated using A_{sat}, leaf size, leaf dry mass and LNC.

3.5.4   Coffee yield estimation

For each site, coffee plants were sampled for coffee yield estimation following a procedure outlined by Meylan (2012). Four plants out of the five coffee plants sampled for other traits were used in this estimation due to time constraints. These measurements were taken between May and July 2013 and represent potential fruiting capacity of each plant. For each plant, every branch was counted. For each branch, every node was counted. On the two bottom-most productive branches, every cherry on each fruiting node was counted. To estimate total fruiting nodes per plant (FN), the percentage of fruiting nodes on the two bottom-most productive branches was multiplied with the total nodes per plant (Equation 3). To estimate total cherries per plant, the estimated total fruiting nodes per plant was multiplied with the average number of cherries per fruiting nodes, as counted on the two bottom-most productive branches (Equation 4).

$$FN = \text{fruiting node subsample (\%) * total nodes per plant} \qquad (3)$$
$$\text{Cherries per plant} = FN * \text{cherries per fruiting node average} \qquad (4)$$

3.6　Soil metrics

Soil samples were collected once (static point sampling) during the month of May and were timed to avoid the influence of recent rainfall events and fertilizer application. Surface litter was cleared beneath each coffee plant sampled. An aggregate soil sample was collected approximately 20 cm from the base of each coffee plant (n= 15 per treatment per site) to a depth of 20 cm in order to capture the coffee rooting zone (DaMatta et al, 2007; Munroe, 2013). At the Llano Bonito site, the slope was exceptionally steep, which required soil sample collection to occur on the upslope side to correspond to sites of fertilizer application (personal communication, Farm Manager, 2013). Soil samples were placed in sealed plastic bags until further subsampling.

A subsample of approximately 5 g from each soil sample bag was weighed and this wet weight recorded. This subsample was then oven-dried at 105°C for at least 72 hours, after which the dry weight was immediately recorded to determine soil moisture content. The remaining fresh soil was divided into two separate portions. One portion was placed directly into the freezer at a temperature of -4°C until further analysis. One portion was air-dried in a ventilated laboratory space for 2-4 weeks, after which the sample was ground with a mortar and pestle and sieved to less than 2 mm.

At the time of analysis for available soil phosphorus (P), 4 g of air-dried and sieved soil was placed in Erlenmeyer flasks, to which 20 mL of Brays 1 was added, shaken for 5 minutes, and filtered through #1 Whatman filter paper into glass vials. The frozen soil subsamples, used to determine available soil N, were allowed to defrost immediately prior to analysis, from which a subsample of 2 g was placed in Erlenmeyer flasks and 20 mL of potassium chloride (KCl) was added. This solution was shaken for 30 minutes and then filtered through #1 Whatman filter paper into glass vials. Each vial was then run through a flow injection analyzer (Lachat QuikChem) to determine orthophosphates, and ammonium and nitrates colourimetrically. It is important to note that although static point sampling to determine soil ammonium and soil nitrates is not ideal, as it cannot capture annual variability due to climatic influences, this data was used as a proxy for available soil N. To determine total soil N and total soil C, approximately 70 to 80 mg of air-dried and sieved soil was weighed on a microbalance and

placed in sealed tin capsules. Total soil N and total soil C were then measured with a CHN analyzer (Thermo Flash 2000, Thermo Scientific, MA, USA).

3.7   Statistical analysis

Normality of data was tested using the Shapiro-Wilk test. Data was log-transformed where necessary to achieve normality. Using Lund's Critical Values, outliers were removed from the dataset. Phenotypic plasticity index was calculated following the procedure proposed by Valladares et al (2000), using the mean maximum and mean minimum trait values. Pearson's correlation coefficients were used to assess linear correlation between coffee leaf traits (Table 1) across the entire dataset, incorporating both induced gradients together in order to assess overall trends in coffee leaf trait co-variation. As some environmental variables did not meet the assumption of normality even after transformation, Spearman's rank correlation coefficients were used to assess correlations between coffee leaf traits and environmental variables (Table 1) across the entire dataset to capture leaf trait and environment relatedness. Using regression analyses, the slope of the linear correlation was compared across treatments for some variables. A one-way analysis of variance (ANOVA) was used to test differences in leaf trait variables between i) shade tree biodiversity treatments, ii) research sites, and iii) FS and shade treatments at each research site. Statistical analyses were performed using STATISTICA 8 (StatSoft Inc. Tulsa, OK, USA) and SAS version 9.2 (SAS Institute Inc. Cary, NC, USA). A type I error rate was set at 0.05 for most statistical tests, although results with significance values at 0.10 and 0.01 are also presented.

Table 1. List of measured coffee plant and environmental variables with corresponding short forms and units.

Variable	Abbreviation	Units
Productivity Traits		
Estimated fruiting nodes	FN	per plant
Estimated cherries	Cherries	per plant
Physiological Traits		
Photosynthesis under saturating irradiance	A_{sat}	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
Mass-based photosynthesis under saturating irradiance	A_{mass}	$\mu mol\ CO_2\ g^{-1}\ s^{-1}$
Stomatal conductance	G_s	$mol\ H_2O\ m^{-2}\ s^{-1}$
Dark respiration	Rd	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
Morphological Traits		
Aboveground biomass	Aboveground biomass	$kg\ plant^{-1}$
Leaf dry mass	Leaf dry mass	g
Leaf size	Leaf size	cm^2
Specific leaf area	SLA	$mm^2\ mg^{-1}$
Specific leaf mass	SLM	$mg\ mm^{-2}$
Leaf dry matter content	LDMC	$mg\ g^{-1}$
Leaf thickness	Leaf thickness	mm
Leaf nitrogen concentration	LNC	$mg\ g^{-1}$
Leaf nitrogen content	LNContent	$mg\ leaf^{-1}$
Photosynthetic nitrogen use efficiency	PNUE	$\mu mol\ C\ g^{-1}\ N$
Environmental Characteristics		
Total light transmittance	Total light transmittance	%
Total diffuse light	Total diffuse light	%
Canopy openness	Canopy openness	%
Soil moisture	Soil moisture	%
Available soil nitrogen	Available soil N	$mg\ kg^{-1}$
Available soil phosphorus	Available soil P	$mg\ kg^{-1}$
Total soil nitrogen	Total soil N	$mg\ kg^{-1}$
Total soil carbon	Total soil C	$mg\ kg^{-1}$

Chapter 4
Results

4.1 Intraspecific phenotypic plasticity

The calculated phenotypic plasticity of each trait across the shade tree biodiversity gradient and the climato-edaphic gradient is presented in Table 2. Across the shade tree biodiversity gradient, productivity traits (FN and cherries) showed 350% and 465% greater plasticity than physiological or morphological traits, respectively. Physiological traits (A_{sat}, A_{mass}, G_s and Rd) still had relatively high plasticity index values, suggesting a large amount of variability across the selected study sites. The mean plasticity index for physiological traits (0.224) was greater than the mean morphological traits plasticity index (0.169). Among the physiological and morphological traits, A_{mass} (0.275) and leaf size (0.342), respectively, were the most plastic across the shade tree biodiversity gradient.

Across the climato-edaphic gradient, productivity traits (FN and cherries) were again more plastic compared to physiological and morphological traits, having 130% and 269% greater plasticity compared to mean physiological and mean morphological traits, respectively. Similarly, physiological traits (A_{sat}, A_{mass}, G_s and Rd) were more plastic on average compared to mean morphological traits (0.526 and 0.253, respectively). Of these two groupings, G_s (0.780) and leaf dry mass (0.446) were the most plastic across the climato-edaphic gradient.

4.2 Coffee trait correlations across all sites

Pearson's correlation coefficients for all leaf traits are presented in Appendix 1. SLA had significant negative correlations with other morphological traits; as SLA increased, LDMC decreased ($r = -0.60$; $p < 0.0001$) and leaf thickness decreased ($r = -0.34$; $p < 0.0001$) (Figure 4). Furthermore, as SLA increased, LNC tended to increase ($r = 0.38$; $p < 0.0001$), A_{mass} tended to increase ($r = 0.19$; $p = 0.0238$) (Figure 4) and G_s tended to decrease ($r = -0.19$; $p = 0.0198$). There was an insignificant positive correlation between SLA and PNUE ($r = 0.11$; $p = 0.2049$).

A significant positive relationship existed between A_{sat} and G_s ($r = 0.72$; $p < 0.0001$). G_s was also significantly positively related to coffee leaf thickness ($r = 0.38$; $p < 0.0001$). A_{mass} tended to

Table 2. Index of phenotypic plasticity (PI) of productivity, physiological and morphological traits of coffee plants grown (A) across the shade tree biodiversity gradient and (B) across the climato-edaphic gradient. Values in parentheses are the calculated mean plasticity index values for each trait group.

Traits	(A) PI	(B) PI
Productivity Traits		
FN (per plant)	0.745	0.680
Cherries (per plant)	0.826	0.683
	(0.786)	(0.681)
Physiological Traits		
A_{sat} ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	0.238	0.441
A_{mass} ($\mu mol\ CO_2\ g^{-1}\ s^{-1}$)	0.275	0.385
G_s ($mol\ H_2O\ m^{-2}\ s^{-1}$)	0.256	0.780
Rd ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	0.125	0.498
	(0.224)	(0.526)
Morphological Traits		
Aboveground biomass ($kg\ plant^{-1}$)	0.120	0.213
Leaf dry mass (g)	0.314	0.446
Leaf size (mm^2)	0.342	0.212
SLA ($mm^2\ mg^{-1}$)	0.067	0.146
SLM ($mg\ mm^{-2}$)	0.089	0.133
LDMC ($mg\ g^{-1}$)	0.076	0.163
Leaf thickness (mm)	0.060	0.145
LNC ($mg\ g^{-1}$)	0.037	0.199
LNContent ($mg\ leaf^{-1}$)	0.340	0.441
PNUE ($\mu mol\ CO_2\ g^{-1}\ N$)	0.246	0.436
	(0.169)	(0.253)
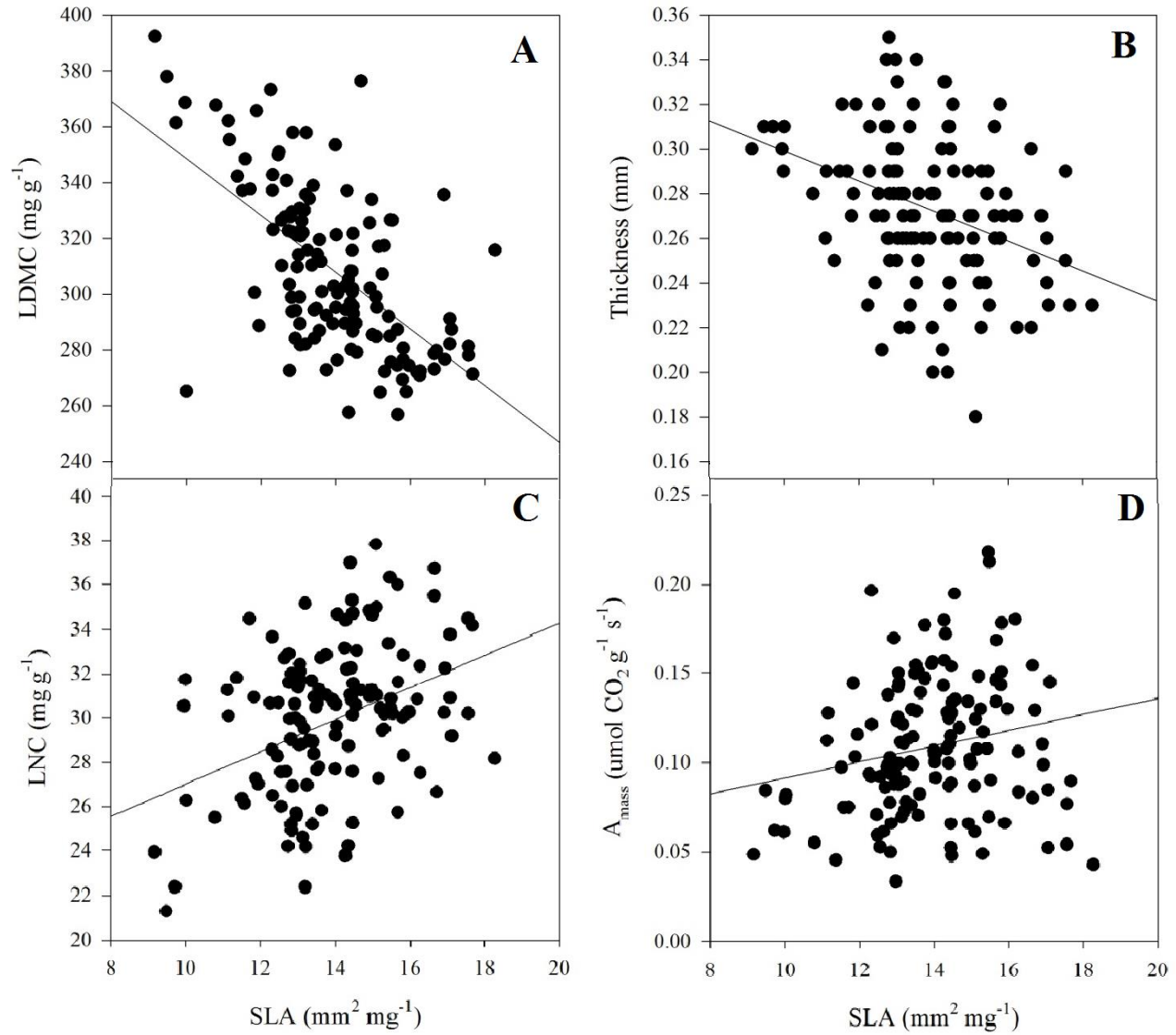
Figure 4. Significant correlations between specific leaf area (SLA) (mm^2 mg^{-1}) and (A) leaf dry matter content (LDMC) (mg g^{-1}); (B) leaf thickness (mm); (C) leaf nitrogen concentration (LNC) (mg g^{-1}), and (D) mass-based photosynthesis (A_{mass}) (μmol CO_2 g^{-1} s^{-1}) across all treatments and research sites. Linear correlations were fitted to the data. [LDMC (r= -0.60; p<0.0001), n= 142; leaf thickness (r= -0.34; p<0.0001), n= 146; LNC (r= 0.38; p<0.0001), n= 146; A_{mass} (r= 0.19; p= 0.0238), n =146].

increase as LDMC decreased (r= -0.38; p<0.0001), as leaf thickness increased (r= 0.15; p= 0.0648), and as LNC increased (r= 0.20; p= 0.0161) (Figure 5).

Coffee productivity traits did not share many significant relationships with other coffee plant traits. FN and cherries had a significant negative correlation with leaf size (r= -0.18; p= 0.0556; and r= -0.17; p= 0.0783, respectively), with leaf dry mass (r= -0.25; p= 0.0082; and r= -0.18; p= 0.0634, respectively), and with LNContent (r= -0.24; p= 0.0116; and r= -0.19; p= 0.0466, respectively). These productivity traits did not share any significant correlations with other physiological traits, including A_{sat}.

4.3 Environmental gradients

Spearman's rank correlation coefficients for coffee plant traits to all environmental variables are presented in Appendix 2. It is important to note that these environmental variables represent above and belowground conditions for the particular sampling day. Since these variables were measured at one time during the year due to time constraints, they cannot be assumed to represent conditions throughout the growing season. This must strongly be considered when analysing aboveground traits, as shade trees are pruned at different times of the year, as well as belowground traits, as Costa Rica experiences a wet season (May - November) and a dry season (December - April).

Coffee productivity traits were related to both incoming light variables and some soil characteristics. Both FN and cherries had a significant positive relationship with total light transmittance (r= 0.47; p<0.0001 and r= 0.52; p<0.0001, respectively). FN had a significant positive relationship with available soil N (r= 0.17; p= 0.0847).

SLA did not share significant relationships with the measured environmental gradients. However, coffee leaf size was significantly negatively related to total light transmittance (r= -0.27; p= 0.0010) (Figure 6). This variable was positively related, yet insignificantly so, to soil available N and soil moisture. LDMC had significant negative correlations with soil moisture (r= -0.39; p<0.0001) (Figure 7) and soil available N (r= -0.41; p<0.0001) (Figure 8), and an insignificant negative correlation with the measured light variables.
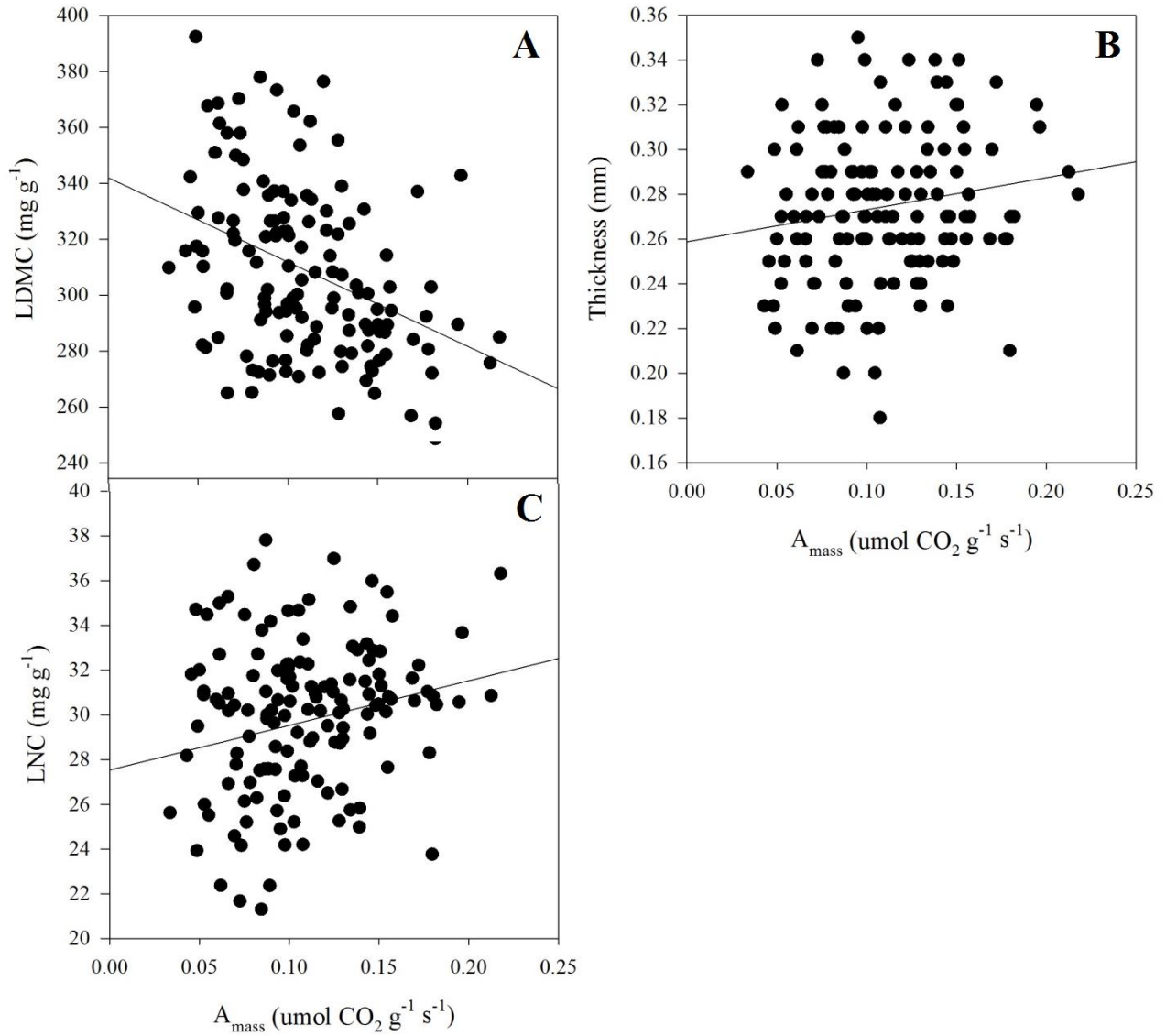
Figure 5. Significant correlations between mass-based photosynthesis (A_{mass}) (μmol CO2 g^{-1} s^{-1}) and (A) leaf dry matter content (LDMC) (mg g^{-1}); (B) leaf thickness (mm); (C) leaf nitrogen concentration (LNC) (mg g^{-1}) across all treatments and research sites. Linear correlations were fitted to the data. [LDMC (r= -0.38; p<0.0001), n= 145; leaf thickness (r= 0.15; p= 0.0648), n= 150; LNC (r= 0.20; p= 0.0161), n= 150].
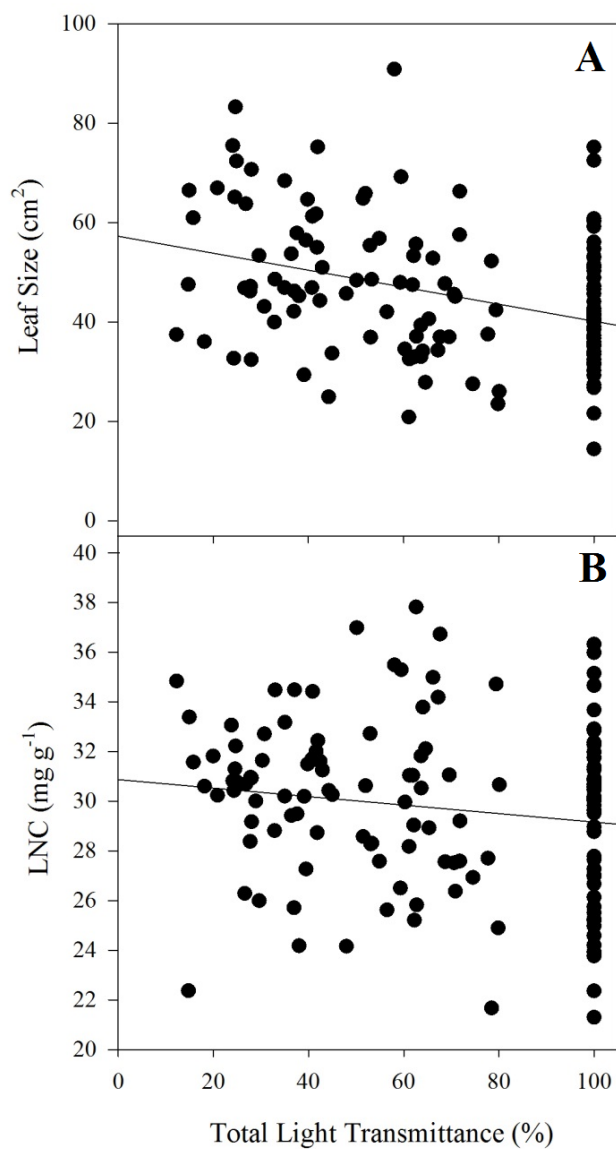
Figure 6. Significant Spearman's correlations between total light transmittance (%) and (A) leaf size (cm^{-2}) and (B) leaf nitrogen concentration (LNC) (mg g^{-1}) across all treatments and research sites. Linear correlations were fitted to the data. [Leaf size (r= -0.27; p= 0.0010), n= 149; LNC (r= -0.14; p= 0.0927), n= 149].
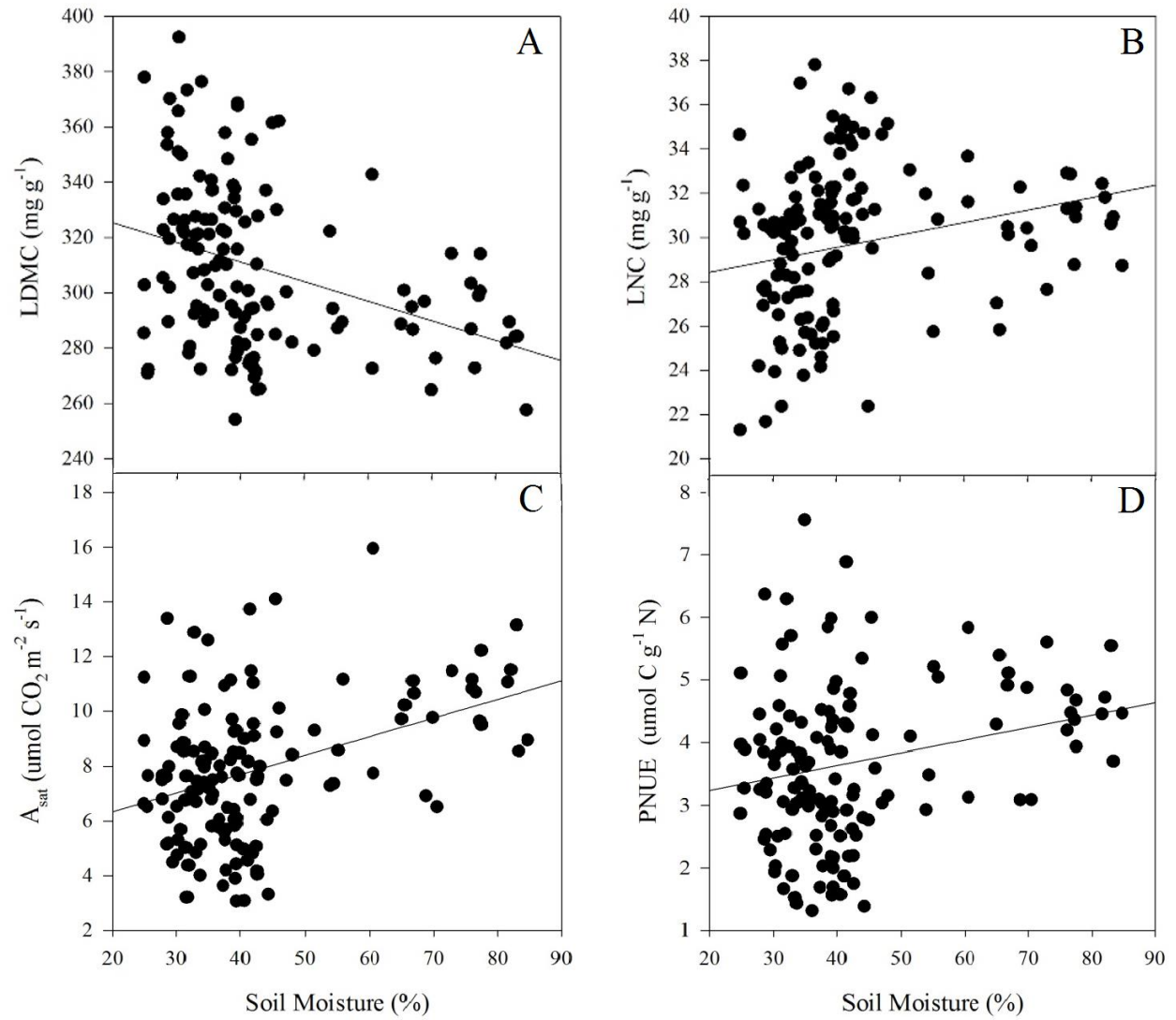
Figure 7. Significant Spearman's correlations between soil moisture (%) and (A) leaf dry matter content (LDMC) (mg g^{-1}); (B) leaf nitrogen concentration (LNC) (mg g^{-1}); (C) photosynthesis under saturating irradiance (A$_{sat}$) (µmol CO_2 m^{-2} s^{-1}) and (D) PNUE (µmol C g^{-1} N). Linear correlations were fitted to the data. [LDMC (r= -0.39; p<0.0001), n= 143; LNC (r= 0.30; p= 0.0002), n= 143; A$_{sat}$ (r= 0.29; p= 0.0005) n= 144, PNUE (r= 0.14; p= 0.0971, n= 148].
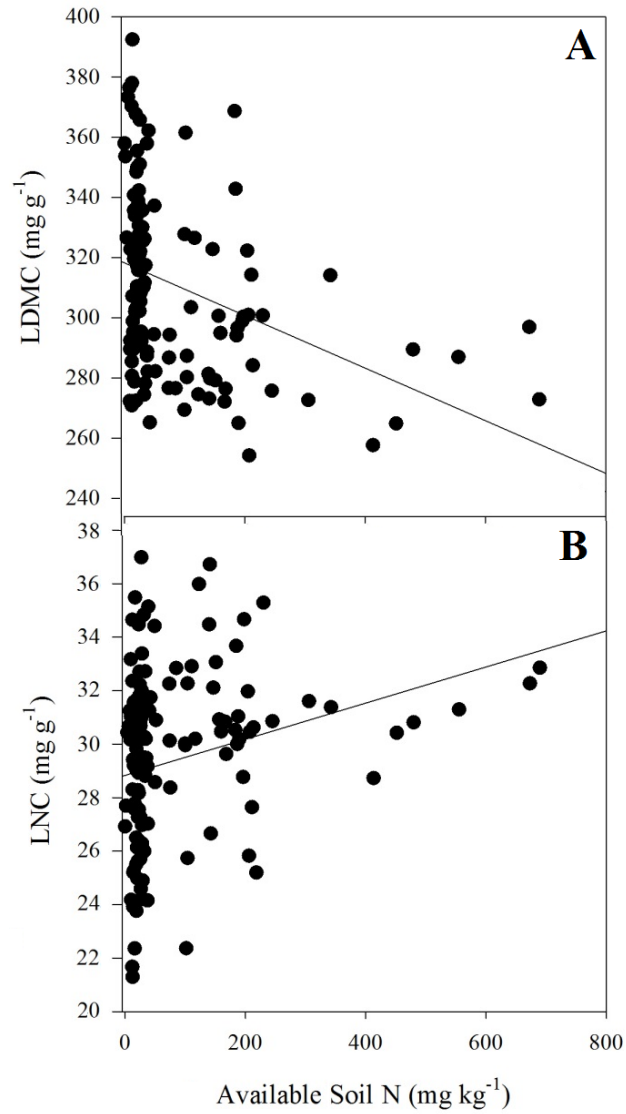
Figure 8. Significant Spearman's correlations between available soil nitrogen (available soil N) (mg kg^{-1}) and (A) leaf dry matter content (LDMC) (mg g^{-1}) and (B) leaf nitrogen concentration (LNC) (mg g^{-1}) across all treatments and research sites. Linear correlations were fitted to the data. [LDMC (r= -0.41; p<0.0001), n= 134; LNC (r= 0.31; p= 0.0002), n= 139].

Similar to coffee leaf size, LNC and LNContent had significant negative correlations with total light transmittance (r= -0.14; p= 0.0927 and r= -0.40; p<0.0001, respectively) (Figure 6). LNC and LNContent also had significant positive correlations with soil moisture (r= 0.38; p<0.0001 and r= 0.27; p= 0.0010, respectively) (Figure 7) and soil available N (r= 0.31; p= 0.0002 and r= 0.14; p= 0.0980) (Figure 8).

Though A_{sat} and PNUE did not share any significant linear relationships with incoming light variables, they were significantly correlated with soil moisture (r= 0.29; p= 0.0005 and r= 0.14; p= 0.0971, respectively) (Figure 7). G_s and Rd were significantly correlated with total light transmittance (r= 0.20; p= 0.0162 and r= 0.19; p= 0.0228, respectively), as well as with soil moisture (r= 0.20; p= 0.0147 and r= 0.30; p= 0.0002).

4.4   Coffee trait correlations across management gradients

Comparing traits across shade tree biodiversity treatments, FN and cherries exhibited significantly higher means in the FS treatment (318 ± 51.3 per plant and 1037 ± 180.8 per plant, respectively) compared to any of the shade treatments (Table 3). Coffee leaf size was significantly greater (p= 0.05) in both the shade*2 and shade*3 treatments compared to the FS treatment (shade*2= 55.9 ± 2.82 cm^2; shade*3= 52.6 ± 4.93 cm^2; FS= 36.8 ± 2.45 cm^2). Leaf size in treatment shade*1 was not significantly greater than in FS, but it was significantly lower than in shade*2 (p= 0.05). There were no significant differences in SLA, SLM, leaf thickness, or LDMC across the treatment types, similar to observations for LNC and PNUE. There was no significant difference in A_{sat} between the shade*2, shade*3 and FS treatments. However, A_{sat} in the shade*2 treatment was significantly greater (p= 0.05) compared to the shade*1 treatment (8.49 ± 0.589 μmol CO_2 m^{-2} s^{-1} and 6.46 ± 0.446 μmol CO_2 m^{-2} s^{-1}, respectively). There were no significant differences between treatments in any of the other measured physiological traits (A_{mass}, G_s, Rd).

As previously mentioned, FN and leaf size had a significant negative correlation across all treatments and research sites (r= -0.18; p= 0.0556). These variables correlated on pooled data but showed different relationships when categorized into shade tree biodiversity treatments. In both the FS and shade*1 treatments, the negative correlation was upheld (r= -0.18; p= 0.5833 and r= -0.67; p= 0.0177, respectively), although only the correlation in the shade*1 treatment was

Table 3. One-way analysis of variance of productivity, physiological and morphological traits of coffee plants grown under varying shade tree biodiversity (FS= full sun; shade*1= one shade tree; shade*2= two shade trees; shade*3= three shade trees). Mean and standard error are presented. Values denoted with different letters are significantly different at $p<0.05$.

	FS	Shade*1	Shade*2	Shade*3
Productivity Traits				
FN (per plant)	318 ± 51.3 a	148 ± 26.5 b	81 ± 15.2 b	146 ± 41.4 b
Cherries (per plant)	1037 ± 180.8 a	421 ± 110.6 b	180 ± 51.0 b	312 ± 85.3 b
Physiological Traits				
A_{sat} (μmol CO_2 m^{-2} s^{-1})	7.42 ± 0.548 ab	6.46 ± 0.446 b	8.49 ± 0.589 a	7.58 ± 0.742 ab
A_{mass} (μmol CO_2 g^{-1} s^{-1})	0.10 ± 0.009 a	0.09 ± 0.007 a	0.12 ± 0.009 a	0.11 ± 0.011 a
G_s (mol H_2O m^{-2} s^{-1})	0.18 ± 0.035 a	0.21 ± 0.063 a	0.13 ± 0.046 a	0.16 ± 0.019 a
Rd (μmol CO_2 m^{-2} s^{-1})	-1.07 ± 0.076 a	-1.03 ± 0.060 a	-0.99 ± 0.105 a	-1.11 ± 0.071 a
Morphological Traits				
Aboveground biomass (kg plant^{-1})	0.4 ± 0.03 a	0.4 ± 0.02 a	0.4 ± 0.02 a	0.4 ± 0.02 a
Leaf size (cm^2)	36.8 ± 2.45 c	41.0 ± 3.38 bc	55.9 ± 2.82 a	52.6 ± 4.93 ab
Leaf dry mass (g)	0.3 ± 0.02 b	0.3 ± 0.03 ab	0.4 ± 0.02 a	0.4 ± 0.03 ab
SLA (mm^2 mg^{-1})	13.6 ± 0.52 a	13.3 ± 0.46 a	14.0 ± 0.51 a	14.5 ± 0.48 a
SLM (mg mm^{-2})	0.08 ± 0.004 a	0.08 ± 0.003 a	0.07 ± 0.003 a	0.07 ± 0.002 a
Leaf thickness (mm)	0.28 ± 0.009 a	0.26 ± 0.011 a	0.27 ± 0.006 a	0.27 ± 0.011 a
LDMC (mg g^{-1})	375.8 ± 10.81 a	333.9 ± 7.50 a	319.8 ± 10.69 a	308.4 ± 5.71 a
LNC (mg g^{-1})	28.4 ± 1.00 a	28.6 ± 0.85 a	29.2 ± 0.63 a	29.5 ± 0.92 a
LNContent (mg leaf^{-1})	7.8 ± 0.55 b	8.9 ± 0.76 ab	11.8 ± 0.68 a	11.0 ± 1.16 a
PNUE (μmol C g^{-1} N)	3.6 ± 0.28 a	3.0 ± 0.24 a	4.0 ± 0.26 a	3.7 ± 0.32 a

significant. In the remaining shade treatments, very weak and insignificant relationships were found. When leaf size was compared to LDMC across shade tree biodiversity treatments, a consistent negative correlation was observed, though significance was only found in the FS treatment (r= -0.58; p= 0.0249) (Figure 9).

The positive linear correlation between A_{mass} and leaf size across all treatments (r= 0.21; p= 0.0098) was upheld when analyzed at the individual treatment level, though this relationship was only significant in the shade*3 treatment (r= 0.65 p= 0.0090). The slopes of this function for each treatment varied, with the greatest slope in the shade*3 treatment (m= 0.001) (Figure 10). In the shade*2 treatment, the slope of the relationship may be driven by an apparent extreme value. Similarly, the linear correlation between A_{mass} and LNC was positive across the FS, shade*2 and shade*3 treatments, though only significant in the shade*2 and shade*3 (r= 0.58; p= 0.0234 and r= 0.62; p= 0.0142, respectively). The relationship between these two variables was most positive in the shade*2 treatment (m= 0.01) and lowest in the shade*1 treatment (m= 0.00). A_{sat} and G_s had consistently significant positive linear correlations across each treatment (FS: r= 0.60; p= 0.0235; shade*1: r= 0.61; p= 0.0164; shade*2: r= 0.85; p<0.0001; shade*3: r= 0.81; p= 0.0013) (Figure 10). Among each treatment, this function had the most positive slope in the shade*3 treatment (m= 31.43) and the lowest slope in the FS treatment (m= 11.67).

4.5   Correlations across sites and treatments

Estimated FN per plant were significantly higher (p= 0.05) at the high altitude site (FS: 415 ± 68.6 per plant and shade: 372 ± 65.9 per plant) compared to the low altitude shade site (133 ± 30.8 per plant), while the mid-altitude site was not significantly different from either (Table 4). Cherries did not share similar variability, as there were no significant differences between any of the sites or treatments. Furthermore, these results did not show any significant differences between treatment types at individual sites (i.e. between FS and shade).

Coffee leaf size was not significantly different between the research sites or the treatments (Table 4). However, SLA and SLM showed some differences. SLA was significantly higher (p= 0.05) in the high altitude site in both treatments (FS: 15.2 ± 0.49 mm^2 mg^{-1}; shade: 14.8 ± 0.52 mm^2 mg^{-1}) compared to the low altitude (FS: 13.1 ± 0.38 mm^2 mg^{-1}; shade: 13.0 ± 0.38 mm^2 mg^{-1}) and mid-altitude (FS: 13.4 ± 0.26 mm^2 mg^{-1}; shade: 13.5 ± 0.35 mm^2 mg^{-1}) sites.
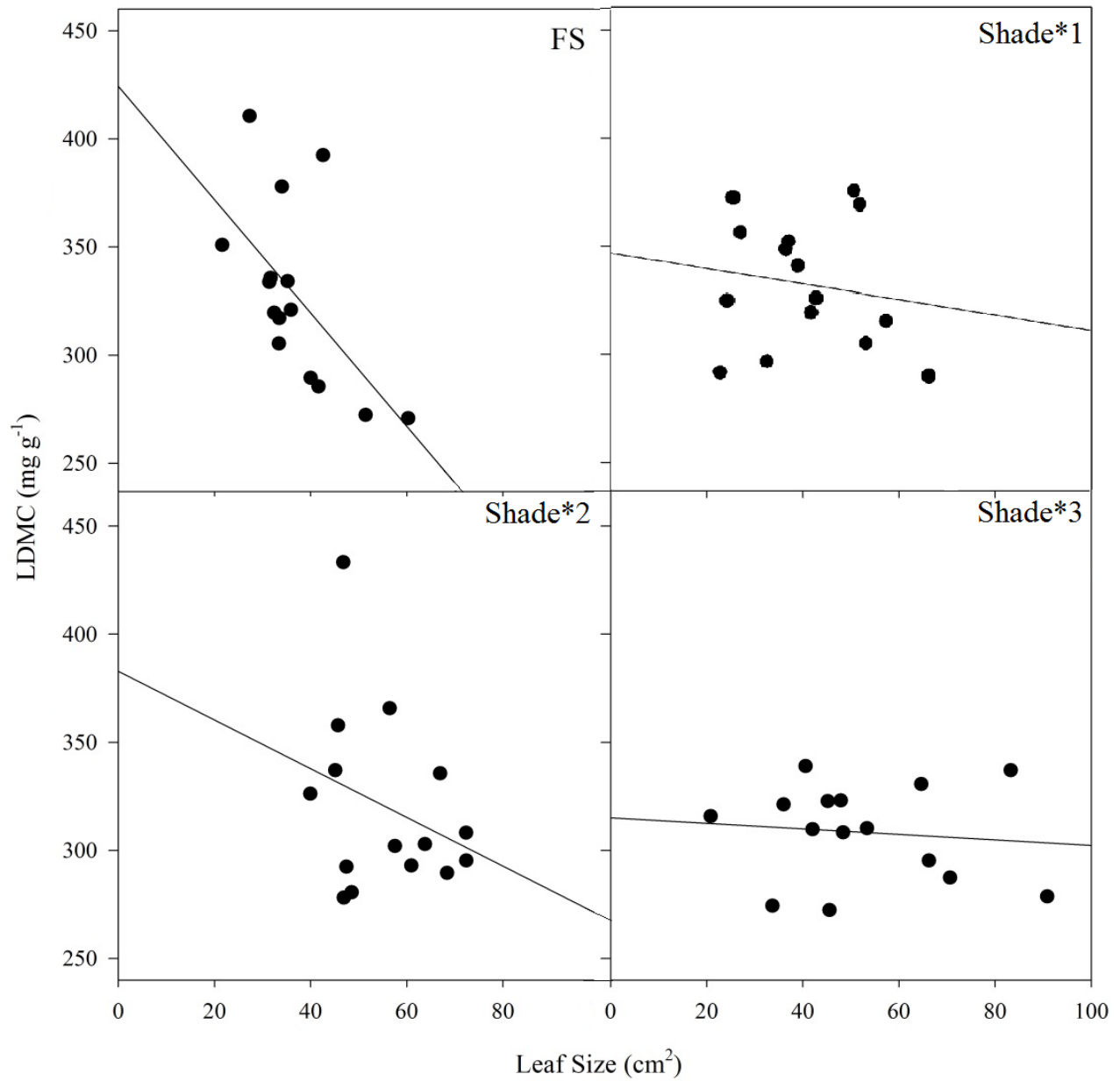
Figure 9. Significant correlations between leaf size (mm^2) and leaf dry matter content (LDMC) (mg g^{-1}) across shade tree biodiversity treatments (FS, shade*1, shade*2, shade*3). Linear correlations were fitted to the data. [FS (r= -0.58; p= 0.0249), n= 15; shade*1 (r= -0.14; p= 0.6296), n= 15; shade*2 (r= -0.30; p= 0.2773), n= 15; shade*3 (r= -0.08; p= 0.7710), n= 15].
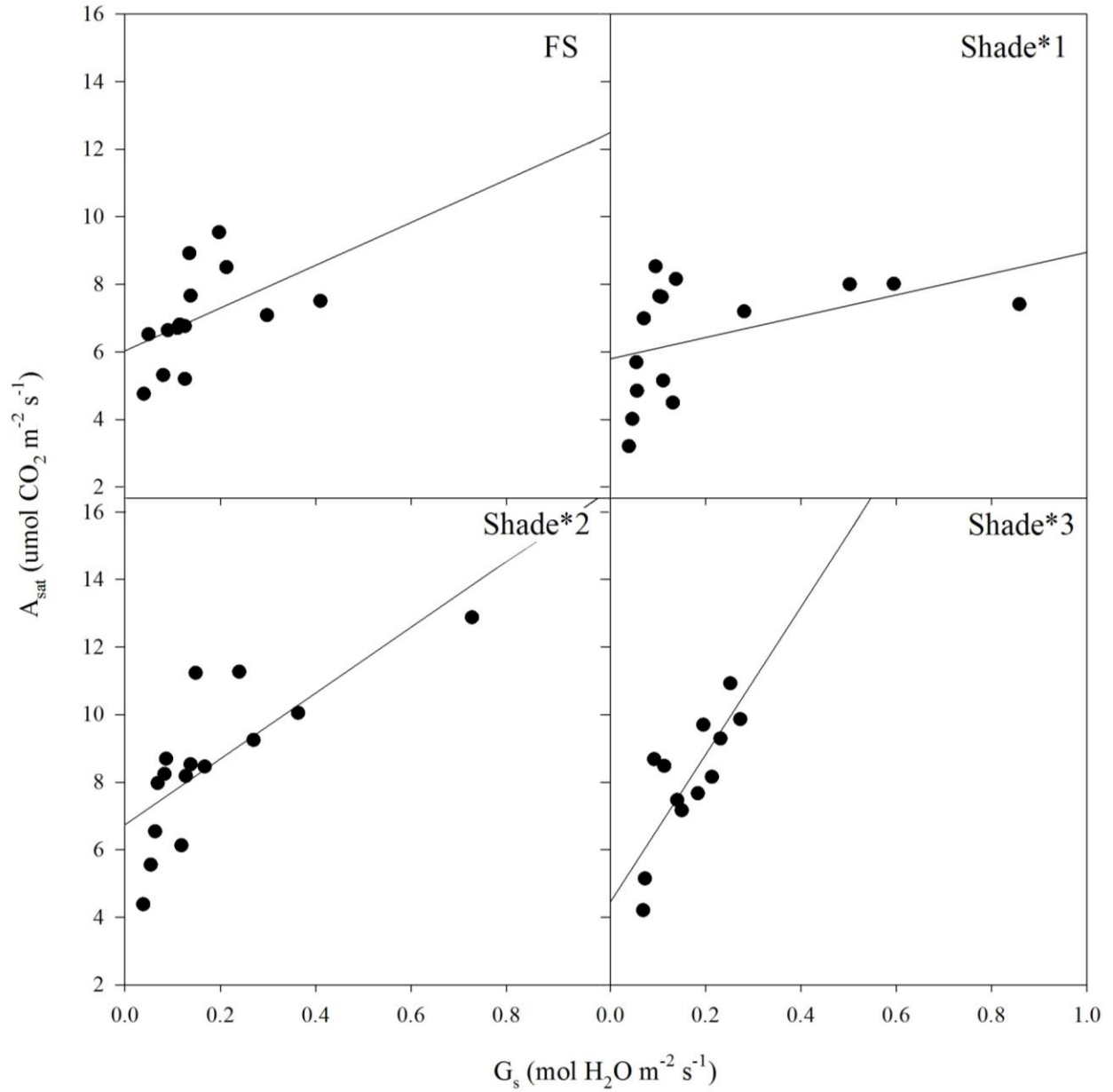
Figure 10. Significant correlations between photosynthesis under saturating irradiance (A_{sat}) (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (G_s) (mol H_2O m^{-2} s^{-1}) across shade tree biodiversity treatments (FS, shade*1, shade*2, shade*3). Linear correlations were fitted to the data. [FS (r= 0.60; p= 0.0235), n= 14; shade*1 (r= 0.61; p= 0.0164), n= 15; shade*2 (r= 0.85; p<0.0001), n= 15; shade*3 (r= 0.81; p= 0.0013), n= 12].

Table 4. One-way analysis of variance of productivity, physiological and morphological traits of coffee plants grown across the three research sites (low altitude; mid-altitude; high altitude) and under different treatments (FS= full sun; shade). Mean and standard error are presented. Values denoted with different letters across all treatments and sites are significantly different at $p<0.05$.

	Low altitude		Mid-altitude		High Altitude	
	FS	**Shade**	**FS**	**Shade**	**FS**	**Shade**
Productivity Traits						
FN (per plant)	275 ± 63.3 ab	133 ± 30.8 b	398 ± 117.0 ab	198 ± 54.0 ab	415 ± 68.6 a	372 ± 65.9 a
Cherries (per plant)	821 ± 220.8 a	427 ± 119.9 a	1300 ± 424.1 a	677 ± 243.1 a	1345 ± 332.2 a	1102 ± 252.0 a
Physiological Traits						
A_{sat} ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	8.22 ± 0.697 ab	6.95 ± 0.362 bc	9.96 ± 0.614 a	9.59 ± 0.504 a	8.34 ± 0.796 ab	5.57 ± 0.527 c
A_{mass} ($\mu mol\ CO_2\ g^{-1}\ s^{-1}$)	0.11 ± 0.010 abc	0.09 ± 0.006 bc	0.13 ± 0.007 a	0.13 ± 0.008 a	0.13 ± 0.014 ab	0.08 ± 0.007 c
G_s ($mol\ H_2O\ m^{-2}\ s^{-1}$)	0.22 ± 0.034 ab	0.21 ± 0.063 bc	0.31 ± 0.030 a	0.24 ± 0.031 ab	0.16 ± 0.023 b	0.07 ± 0.010 c
Rd ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	-1.01 ± 0.090 ab	-1.08 ± 0.059 b	-0.72 ± 0.049 a	-0.77 ± 0.047 a	-0.79 ± 0.084 a	-0.95 ± 0.084 ab
Morphological Traits						
Aboveground biomass (kg plant^{-1})	0.4 ± 0.02 a	0.5 ± 0.03 a	0.5 ± 0.05 a	0.5 ± 0.05 a	0.4 ± 0.02 a	0.5 ± 0.04 a
Leaf size (cm^2)	39.1 ± 2.37 a	48.3 ± 2.81 a	48.8 ± 3.03 a	62.6 ± 5.73 a	40.2 ± 3.10 a	41.8 ± 3.23 a
Leaf Dry mass (g)	0.3 ± 0.02 bc	0.4 ± 0.03 ab	0.4 ± 0.02 ab	0.5 ± 0.04 a	0.3 ± 0.02 c	0.3 ± 0.02 bc
SLA (mm^2 mg^{-1})	13.1 ± 0.38 b	13.0 ± 0.38 b	13.4 ± 0.26 b	13.5 ± 0.35 b	15.2 ± 0.49 a	14.8 ± 0.52 a
SLM (mg mm^{-2})	0.08 ± 0.002 a	0.08 ± 0.002 a	0.08 ± 0.001 a	0.08 ± 0.002 ab	0.07 ± 0.003 b	0.07 ± 0.003 b
Leaf thickness (mm)	0.27 ± 0.012 ab	0.28 ± 0.009 ab	0.29 ± 0.008 a	0.29 ± 0.008 a	0.28 ± 0.006 ab	0.25 ± 0.006 b
LDMC (mg g^{-1})	329.1 ± 6.19 a	322.3 ± 5.47 ab	298.8 ± 4.80 c	286.5 ± 6.33 cd	275.5 ± 2.88 d	303.3 ± 6.56 bc
LNC (mg g^{-1})	26.8 ± 0.71 c	29.1 ± 0.97 bc	30.7 ± 0.67 ab	30.0 ± 0.72 b	31.4 ± 0.77 ab	33.4 ± 0.63 a
LNContent (mg leaf^{-1})	7.9 ± 0.74 c	11.0 ± 0.80 abc	11.2 ± 0.67 ab	14.1 ± 1.39 a	8.1 ± 0.69 bc	9.6 ± 0.87 bc
PNUE ($\mu mol\ C\ g^{-1}\ N$)	4.2 ± 0.37 a	3.1 ± 0.21 ab	4.3 ± 0.24 a	4.3 ± 0.24 a	4.1 ± 0.41 a	2.4 ± 0.21 b

Expectedly, SLM had the opposite pattern, being lowest in the high altitude site (FS and shade: 0.07 ± 0.003 mg mm^{-2}). LDMC varied across research sites and between treatments. LDMC was highest at the low altitude site (FS: 329.1 ± 6.19 mg g^{-1}; shade: 322.3 ± 5.47 mg g^{-1}). At the high altitude site, the FS treatment was the lowest among all treatments (275.5 ± 2.88 mg g^{-1}), and was significantly lower ($p= 0.05$) than the shade treatment of the same site (303.3 ± 6.56 mg g^{-1}).

LNC increased in the treatments as altitude increased, being highest in the high altitude site and lowest in the low altitude site. Although there were no significant differences between treatments within the same site, there was an almost consistent trend of LNC values being higher in the shade treatment compared to FS, though this was not observed at the mid-altitude site where the shade treatment had marginally lower LNC values (FS: 30.7 ± 0.67 mg g^{-1}; shade: 30.0 ± 0.72 mg g^{-1}). PNUE followed the opposite pattern, where rates were lower in the shade treatment within the same site compared to FS. Again, this pattern was not observed at the mid-altitude site, where the values were very similar (FS: 4.3 ± 0.24 µmol C g^{-1} N; shade: 4.3 ± 0.24 µmol C g^{-1} N). The only significant difference observed ($p= 0.05$) was at the high altitude site where FS had higher PNUE values (4.1 ± 0.41 µmol C g^{-1} N) compared to shade (2.4 ± 0.21 µmol C g^{-1} N). Across all sites, PNUE was highest at the mid-altitude site and lowest at the high altitude site.

All of the physiological traits measured (A_{sat}, A_{mass}, G_s, Rd) were highest at the mid-altitude site when FS and shade treatments were considered separately across all sites. At the low and mid-altitude sites, there was no significant difference between the FS and shade treatments, though values were consistently higher in the FS treatments. Significant differences ($p= 0.05$) between FS and shade existed only at the high altitude site, where FS treatments had significantly ($p= 0.05$) higher A_{sat} (FS: 8.34 ± 0.796 µmol CO$_2$ m^{-2} s^{-1}; shade: 5.57 ± 0.527 µmol CO$_2$ m^{-2} s^{-1}), A_{mass} (FS: 0.13 ± 0.014 µmol CO$_2$ g^{-1} s^{-1}; shade: 0.08 ± 0.007 µmol CO$_2$ g^{-1} s^{-1}), and G_s (FS: 0.16 ± 0.023 mol H$_2$O m^{-2} s^{-1}; shade: 0.07 ± 0.010 mol H$_2$O m^{-2} s^{-1}).

Similar to leaf trait means, coffee leaf trait correlations also varied between sites and treatments. When LNC was analysed across sites by treatment, LNC and SLA only had a significant ($p= 0.05$) positive linear correlation in the shaded treatments across all sites (Figure 11). The slope of this function in the shade treatments decreased as altitude increased (low altitude shade: m= 1.40; mid-altitude shade: m= 1.24; high altitude shade: m= 0.62). In the FS treatments, the
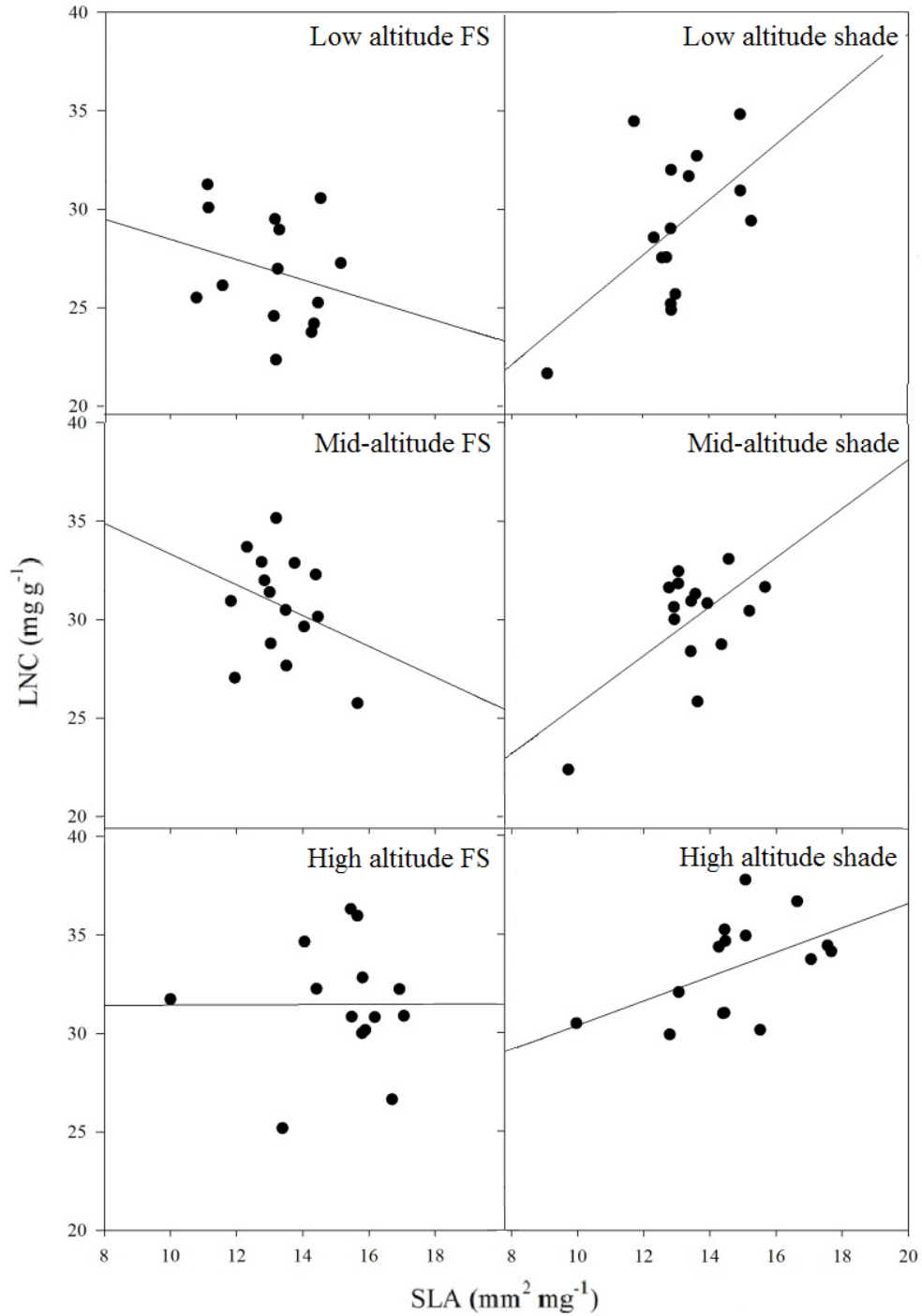
Figure 11. Linear correlation graphs of leaf nitrogen concentration (LNC) (mg g^{-1}) and specific leaf area (SLA) (mm^2 mg^{-1}) across the climato-edaphic gradient at each site (low altitude, mid-altitude and high altitude) and each treatment (FS and shade). Linear correlations were fitted to the data. [Low altitude FS (r= -0.26; p= 0.3672), n= 14; low altitude shade (r= 0.27; p= 0.3488), n= 14; mid-altitude FS (r= -0.31; p= 0.2696), n= 15; mid-altitude shade (r= 0.61; p= 0.0166), n= 15; high altitude FS (r= 0.00; p= 0.9898), n= 14; high altitude shade (r= 0.51; p= 0.0549), n= 15].

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correlation was either negative or very weak, and always insignificant. The relationship between SLA and A_{mass} also varied across sites, having a significant ($p= 0.05$) positive correlation in low altitude FS and mid-altitude shade sites, a significant negative correlation in mid altitude FS, and insignificant relationships at low altitude shade and the high altitude site (Figure 12). There were significant ($p= 0.05$) positive linear correlations between A_{sat} and G_s at all sites and treatments, except for mid altitude shade where correlations were not significant (Figure 13). At both the low altitude and mid-altitude sites, the slope of the function was greater in the FS treatments ($m= 11.14$ and $m= 9.26$, respectively) compared to the shade treatment ($m= 3.93$ and $m= 3.12$, respectively). At the high altitude site, the opposite was found (FS: $m= 29.01$; shade: $m= 47.84$). Furthermore, from the low altitude site to the high altitude site, the slope of the function in both treatments increased.
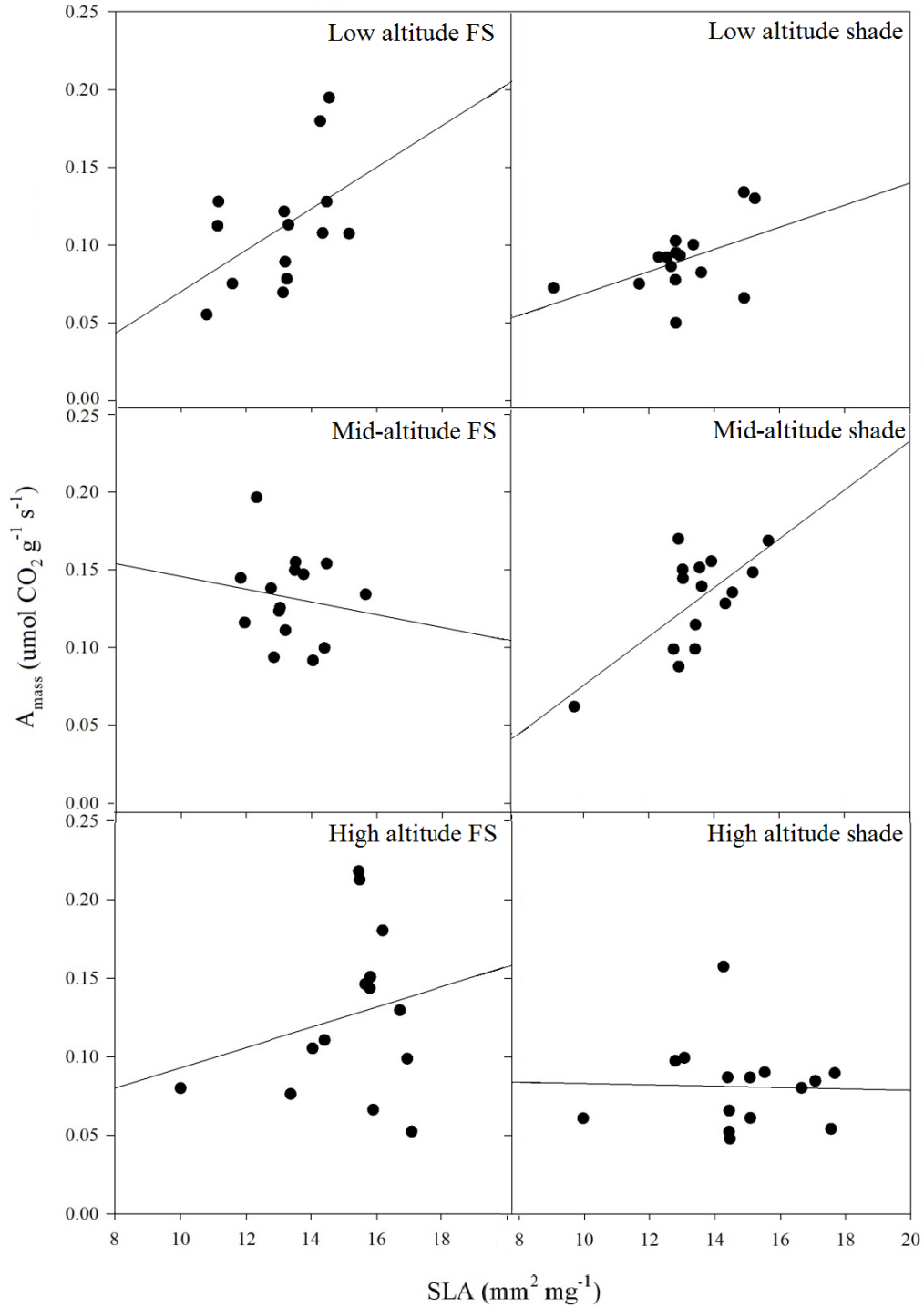
Figure 12. Linear correlation graphs of mass-based photosynthesis (A_{mass}) (μmol CO_2 g^{-1} s^{-1}) and specific leaf area (SLA) (mm^2 mg^{-1}) across the climato-edaphic gradient at each site (low altitude, mid-altitude and high altitude) and each treatment (FS and shade). Linear correlations were fitted to the data. [Low altitude FS (r= 0.51; p= 0.0638), n= 14; low altitude shade (r= 0.39; p= 0.1727), n= 14; mid-altitude FS (r= -0.14; p= 0.6294), n= 15; mid-altitude shade (r= 0.73; p= 0.0019), n= 15; high altitude FS (r= 0.20; p= 0.5026), n= 14; high altitude shade (r= 0.00; p= 0.9903), n= 15].
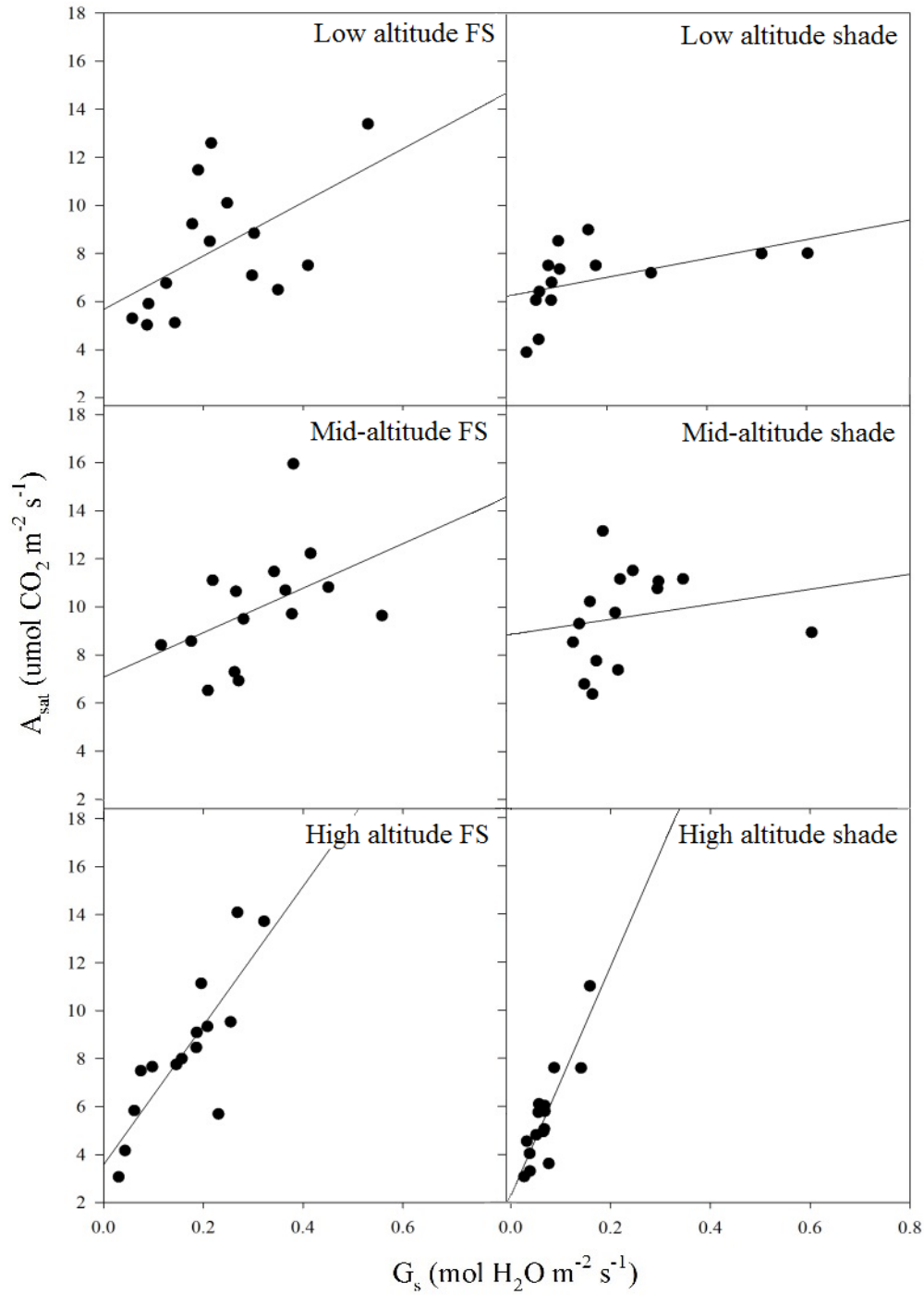
Figure 13. Significant correlations between photosynthesis under saturating irradiance (A_{sat}) (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (G_s) (mol H_2O m^{-2} s^{-1}) across the climato-edaphic gradient at each site (low altitude, mid-altitude and high altitude) and each treatment (FS and shade). Linear correlations were fitted to the data. [Low altitude FS (r= 0.58; p= 0.0222), n= 15; low altitude shade (r= 0.67; p= 0.0086), n= 14; mid-altitude FS (r= 0.47; p= 0.0780), n= 15; mid-altitude shade (r= 0.31; p= 0.2606), n= 15; high altitude FS (r= 0.81; p= 0.0002), n= 15; high altitude shade (r= 0.83; p= 0.0001), n= 15].

Chapter 5
Discussion

5.1 Coffee plant trait plasticity in agroforestry systems

Findings from this study support previous work on trait plasticity in coffee (e.g. Matos et al, 2009; Cavatte et al, 2012) and other plant species (e.g. Valladares et al, 2000; Freschet et al, 2013), where coffee leaf trait plasticity varied given i) shade trees present and ii) the geographical location of the coffee agroforestry system. Drawing from studies on natural populations in temperate regions, Valladares et al (2002 and 2005) suggest that plants characterized as "shade-tolerant" display greater plasticity in morphological traits compared to physiological traits. This distinction was based on the assumption that sun-tolerant species have enhanced physiological plasticity in order to tolerate strong irradiance and avoid photoinhibitory stress; while shade-tolerant species have enhanced morphological plasticity in order to maximize light-harvesting traits (Valladares et al, 2002). Coffee is considered a shade-tolerant species, due to its evolutionary background and current leaf traits (DaMatta, 2004). Therefore, it is expected that coffee morphological traits have higher plasticity than physiological traits. However, my findings do not reflect what has been found in natural plant populations, as the coffee plants in my research plots had greater plasticity among physiological traits compared to morphological traits. Similarly, Matos et al (2009) found that in-field coffee grown with a hedgerow in southeastern Brazil behaved as an intermediary group between sun and shade tolerance, since their leaves did not perform as strictly true sun or true shade leaves.

Coffee leaf acclimation abilities to full sun conditions display their plastic capacity across aboveground light gradients (e.g. DaMatta, 2004; Chaves et al, 2008; Matos et al, 2009). Coffee leaves display plasticity in morphological traits in full sun and shade conditions, including functional plant traits, such as leaf size and leaf thickness (e.g. Gindel, 1962; DaMatta, 2004; Matos et al, 2009). For example, Vaast et al (2008), who conducted a study in the southern lowlands of Costa Rica at an altitude of 640 m.a.s.l., found mean coffee leaf size ranged from 17 cm^2 in full sun up to 45 cm^2 under *Terminalia ivorensis* shade trees. I found similar evidence of plasticity in coffee leaf morphological traits across my shade tree biodiversity gradient, as leaf size and thickness exhibited relatively high plasticity index values. As expected, measured

incoming light, presumably affected by shade tree presence, was strongly correlated with leaf size.

The plasticity index values I calculated also indicate that coffee plant traits are plastic across geographical locations and, subsequently, climato-edaphic conditions. This plasticity reflects the coffee plant's ability to adjust to other microclimatic conditions beyond light transmittance. The plasticity I found corresponds to the variability in coffee leaf traits observed across various study sites in the published literature. For example, I found specific leaf area (SLA) had a plasticity index value of 0.146 across an altitudinal range of 650-1500 m.a.s.l. In the literature, coffee SLA ranged between approximately 8-17 mm^2 mg^{-1} at 554 m.a.s.l. (Righi et al, 2007), while SLA at 650 m.a.s.l. ranged from 12.1-20.8 mm^2 mg^{-1} (Matos et al, 2009). The variability of plant functional traits, like SLA, represents the variability of plant functional ecology across varying climato-edaphic conditions in a managed agricultural system, which has traditionally been documented in natural systems (Garnier and Navas, 2012).

5.2   Co-variation of coffee plant traits across gradients

Given strong correlations between leaf traits in natural plant communities across a range of site conditions (e.g. Marino et al, 2010; Brousseau et al, 2013), as recently described by the worldwide leaf economic spectrum (LES) (Wright et al, 2004), coffee leaf traits were expected to co-vary across the induced biotic and abiotic gradients in the agroforestry systems. Indeed, SLA strongly correlated with many other coffee leaf morphological traits across both the shade tree biodiversity and climato-edaphic gradients. As SLA increased, specific leaf mass (SLM), leaf dry matter content (LDMC) and leaf thickness all decreased, as was expected based on previous findings in natural plant communities (Wilson et al, 1999). These results suggest that although the leaf area was expanding, the volume of the leaf was reduced. Leaf thickness is often associated with the width of the palisade cells for most plant species including coffee (e.g. Givnish, 1988; Matos et al, 2009), which are the sites of leaf chloroplasts necessary for photosynthesis. Thinner coffee leaves have a lower palisade-to-spongy parenchyma ratio, which allows for increased leaf light absorption through increased internal light scattering (Lawren et al, 2006). Therefore, the tendency of coffee leaves to have enhanced SLA with reduced SLM, LDMC and leaf thickness suggests plasticity in the coffee plant to promote the development of resource acquiring traits to maximize light harvesting capabilities. This was indeed observed

through an increase in mass-based photosynthesis (A_{mass}) with increasing SLA across all sampled coffee plants.

Leaf N concentration (LNC) and A_{mass} were positively correlated across all sites and shade tree treatments, thus supporting previous findings from coffee literature (e.g. DaMatta 2004; Reis et al, 2009) and other plant trait literature (e.g. Farquhar et al, 1980; Givnish, 1988), including the LES (Wright et al, 2004). The optimal range of leaf N for growth in field-grown plants is 10-50 mg g^{-1} (Cornelissen et al, 2003). I found that coffee leaf N ranged between 21-38 mg g^{-1} across both gradients, and were, therefore, within the cited optimal range. The positive correlation between LNC and photosynthetic rates is reflective of resource acquisition and presumably due to the important role N plays in the ability of coffee leaves to photosynthesize, since it is i) an integral component of stomatal conductance (G_s), ii) important for some of the pigments and enzymes involved in the Calvin cycle (Terashima and Evans, 1988), and iii) controls the quantum yield of photosynthesis (Lawlor, 1995; Lu and Zhang, 2000).

A positive relationship between photosynthesis and G_s was expected, due to the role of leaf stomata in the transport of CO_2 from the atmosphere into the leaf for the process of photosynthesis (e.g. DaMatta, 2004; Franck and Vaast, 2009). Accordingly, a strong positive correlation was found between light saturated photosynthesis (A_{sat}) and G_s across all sampled coffee plants. However, coffee leaf G_s decreases due to stomatal closure under higher evaporative demands, a reaction of the coffee plant to maintain a favourable water status (DaMatta and Ramalho, 2006). Therefore, it is expected that photosynthesis would decrease in water-limited environments due to the restrictions of lower G_s.

Little work from natural populations compares co-variation in leaf traits to productive traits, though there are similar environmental controls over these relationships. My data provides the opportunity to assess leaf to yield trait relationships and I show that fruiting nodes (FN) and number of cherries indeed were negatively correlated with leaf size across all sampled coffee plants. Within the coffee trait literature, there is variation in the relationship between these traits. It has been observed that coffee grown under field conditions in the southern lowland of Costa Rica tend to produce a greater yield while concurrently having a greater mean leaf size (Vaast et al, 2008). In contrast, coffee grown under field conditions in Viçosa, Brazil had a greater number

of nodes and smaller average leaf area (Campanha et al, 2004). Since enhanced leaf size is characteristic of resource acquisition, trends suggest the classic trade-off between vegetative (leaf) and productive (fruit) growth. Since coffee plants require adequate photosynthetic rates in order to produce and store enough carbohydrates for both productivity traits and vegetative growth (Campanha et al, 2004), heavy flowering in coffee is often not paralleled with sufficient leaf area formation (DaMatta et al, 2007), though the coffee plants in my study had a mean leaf area above the minimum area necessary for the development of good quality cherries (20 cm^2) (Cannell, 1985). However, it is important to note that the correlation between FN and leaf size is dependent on multiple other climatic constraints, and therefore should be interpreted with caution (Funk and Cornwell, 2013).

The number of FN and cherries were negatively correlated with leaf N content (LNContent). Since measurements were obtained between May and June, this time corresponds to the later stages of fruit development (Reis et al, 2009). Nitrogen (N) is an important part of fruit development, since it is part of the many amino acids, proteins, enzymes and pigments required for cherry formation. In order to contribute to yield formation, the protein fraction of N in the leaf is degraded, which occurs both during senescence and at other stages (Hulffaker, 1990). The degraded N is then distributed in the plant (Reis et al, 2009). The negative correlation between FN and cherries with LNContent, therefore, may be explained by the redistribution of N from the leaf to reproductive traits, resulting in lower LNContent values for enhanced productivity. Since this relationship is maintained across all sampled coffee plants, differences in the amount of fertilizer applied to each system (see Chapter 3) are captured, indicating that the relationship between LNContent and productivity is still valid although soil nutrient inputs vary.

It was also found that yield estimates did not significantly correlate with leaf-level photosynthetic rates across either the shade tree biodiversity or climato-edaphic gradient. This is contrary to the coffee literature, which has concluded that lower yields are mainly a result of lower photosynthetic rates (Beer et al, 1998; DaMatta et al, 2007; Franck and Vaast, 2009), due to the coffee plant's carbohydrate limitations. However, these studies must assume that instantaneous leaf-level photosynthetic rates are representative of the entire plant. Scaling leaf-level photosynthesis to the whole plant level requires caution since these processes can vary greatly between coffee leaves (DaMatta, 2004; Araújo, 2006; Marur and Faria, 2006). Although

proper protocol was followed for coffee leaf sampling, it is not unexpected that leaf-level photosynthesis does not correlate to yield metrics at the whole plant level. In order to find strong correlations between yield and photosynthesis variables, further work is needed on whole-plant scale photosynthesis measurements or estimates.

The co-variation of coffee plant traits suggest trends in plant ecological trade-offs. Similarly, the plasticity index values calculated across the shade tree biodiversity and climato-edaphic gradients suggest that coffee leaf traits are variable depending on biotic and abiotic factors. The variability in coffee plant functional traits highlights an important concern for management decisions. The plasticity observed in coffee plant traits in this study and in the literature suggests that coffee plants are capable of acclimation to changing environmental conditions (e.g. DaMatta, 2004; Franck and Vaast, 2009). However, there are limits to these acclimation abilities, as damage to the coffee plant is possible in extreme and harsh conditions when shade trees are not present to moderate these effects (e.g. Lock, 1888; Beer et al, 1998; DaMatta, 2004). Therefore, it is in these harsh conditions that agroforestry is suggested for coffee systems, due to the ability of shade trees to ameliorate the microclimate beneath their canopies (Siles et al, 2010). It is expected that global climate patterns will change in the future and that climate-sensitive coffee will be negatively affected (Davis et al, 2012). Therefore, agroforestry has been suggested as an efficient and inexpensive mitigation method for smallholder coffee farmers (DaMatta et al, 2007; Lin, 2007).

5.3   Coffee traits and shade tree management

Coffee plant traits showed high plasticity in response to, or indeed to cope with, environmental variables, including various light levels ranging from full sun to high levels of shade, as well as gradations of soil nutrient and water status. These contrasting environments were operationalized in this study to test whether coffee plasticity was induced by shade tree diversity presence in agroforestry systems. It is important for agroforestry systems to understand how these relationships translate into management regimes in order to optimize coffee performance under variable environmental conditions. Therefore, each coffee trait and some of the established correlations were categorically compared across agroforestry management type: full sun (FS); coffee with *E. poeppigiana* (shade*1); coffee with *E. poeppigiana* and *T. amazonia* (shade*2); and coffee with *E. poeppigiana*, *T. amazonia* and *C. eurycyclum* (shade*3).

I demonstrate that SLA and leaf size were highly important variables in many trait-to-trait correlations across all sites. Leaf size in the shade*3 treatment was significantly greater than the FS treatment. Concurrently, leaf size significantly increased with decreasing light availability, which reflects the literature that has found that coffee sun leaves are generally smaller in area compared to shade leaves (e.g. Campanha et al, 2004; Vaast et al, 2008; Matos et al, 2009; Cavatte et al, 2012). In reduced light conditions under shade trees, coffee leaves acclimate to promote photosynthesis under low irradiance through the increased photosynthetic surface area of the leaf (Boardman, 1977), which has been previously reported for coffee leaves (Hollies, 1967; Fahl et al, 1994; Franck, 2005). This adaptation to low light levels is indicative of resource-acquiring capabilities (Wright et al, 2004), as shown in natural tree populations (Grassein et al, 2010; Freschet et al, 2013), where intraspecific response appears to exploit variable resource availability.

Total light transmission below the shade tree canopy ranged from 15 to 100% across the shade tree biodiversity gradient. This range of light transmission, however, did not correlate with the number of shade tree species present. Instead, it may have been influenced by distance of the sampled coffee crop to the shade tree (within the standardized 3 m radius) and shade tree canopy architecture (as affected by species type and pruning regime), which may affect the quality of light transmitted. This suggests that management should strongly consider shade tree species selection and pruning schedule due to their large influence on incoming light variables (Beer et al, 1998). Previous research has found that, in the southern lowlands of Costa Rica at an altitude of 640 m.a.s.l., coffee grown under the shade of *Terminalia* had high rates of vegetative growth with mean leaf size of 45 cm^2, attributed to its denser shade; however, coffee grown under *E. poeppigiana* had growth similar to full sun coffee with mean leaf size of 30 cm^2, attributed to its lower and irregular canopy (Vaast et al, 2008). I found that leaf size almost consistently increased with increasing type of shade trees present. However, the coffee leaves under exclusively *E. poeppigiana* in shade*1 were smaller in size, thus not following this trend but reflective of observations by Vaast et al (2008). Similarly, the inconsistent shading of the *E. poeppigiana* trees due to pruning management may cause this pattern. This may cause the shade quality to not be consistent in comparison with other shade treatments, including *T. amazonia* and *C. eurycyclum*, whose shade is larger and more uniform (Campbell, 2012).

Coffee leaf size and LNC were both significantly correlated with A_{mass} across all of the treatments. The magnitude of the correlations between these important leaf physiological and morphological traits varied across the shade tree biodiversity categories. This is not unexpected, as I showed strong relationships between these coffee plant traits and environmental variables. Therefore, the variability in the correlations between leaf size and LNC with A_{mass} is related to differences in plant ecological trade-offs due to changes in environmental conditions. This has been observed in natural plant communities as well, where the plant ecological trade-offs observed between plant communities are not always observed within communities, often dependent on climatic constraints (Funk and Cornwell, 2013). Since both enhanced leaf size and LNC are characteristically resource-acquiring traits (e.g. Givnish, 1988; Freschet et al, 2013), their greater influence on A_{mass} as observed in the shade treatments reflects an environment where resources are limited, likely light variables due to the increased shade tree species richness. Furthermore, I found that LNC and available soil N were positively related. Since shade trees may contribute to N availability in the soil via dinitrogen (N_2)-fixation and direct and indirect transfer (Munroe and Isaac, 2013), increased shade tree species richness may have enhanced available soil N, contributing to enhanced LNC and subsequently A_{mass}, although I did not determine exact pathways of soil N transfer in this study. Therefore, although shade trees may limit aboveground light resources, my study demonstrates that there may be other complementary or facilitative influences above or belowground that promotes coffee leaf physiological and morphological traits, which resulted in few significant differences between the FS and shade treatments.

Across all sampled coffee plants, A_{sat} did not share a strong correlation with incoming light. However, a non-linear correlation was expected between A_{sat} and light variables, reflective of the literature citing a threshold of optimum light penetration approximately between 50% and 80% (e.g. Carelli et al, 1999; Matos et al, 2009; Campbell, 2012). The discrepancy may be the result of the methodology chosen in quantifying the select light variables. Hemispherical photography was selected in order to characterize the study site, whereas another method may have been better suited for direct comparison. Given more accurate light measurements, I would expect a strong non-linear relationship between A_{sat} and incoming light variables.

The relationship between A_{sat} and G_s also varied across the different treatments. Franck and Vaast (2009) report that the slope of this function decreased linearly as shade increased when the only variable was artificial shade. However, in this study, the slope of the function increases almost linearly as shade tree biodiversity increases, indicating that there is less stomatal opening required for higher rates of photosynthesis. Since soil moisture increased with increasing shade tree presence, and stomatal aperture is generally greater in shade treatments due to higher soil moisture and reduced evaporative demands (DaMatta et al, 2002), G_s is not limiting photosynthetic rates in the shade treatments. Instead, incoming light through the shade trees limited photosynthesis. However, this study found that A_{sat} was not statistically different between FS or any of the shade treatments, indicating that at an altitude of 650 m.a.s.l., shade tree presence does not inhibit physiological processes. When this is the case, previous studies often use lower G_s values as an explanation (e.g. Kumar and Tieszen, 1980; Kanechi et al, 1995; Freitas, 2000; Carelli et al, 2001; Paiva et al, 2001), which was observed here.

FN and cherries were significantly higher in the FS treatment compared to any of the shade treatments. There have been numerous studies that show increasing FN and yield with increasing light availability (e.g. Campanha et al, 2004; DaMatta, 2004; Franck and Vaast, 2009). Findings across the shade tree biodiversity gradient conformed to these established relationships, as FN and cherry counts significantly increased with total light transmittance, as measured with hemispherical photography. In this case, the aboveground interspecific competition for incoming light may have led to trade-offs between vegetative and reproductive growth, which can result in conflicting resource allocation within the coffee plant (Thomaziello et al, 2000; Campanha et al, 2004). It has also been proposed that this trend is due to higher carbon assimilation rates for the entire plant in full sun (Beer et al, 1998; DaMatta et al, 2007) and more nodes formed per branch with more cherries per node (Montoya et al, 1961; Castillo and López, 1966).

Haggar et al (2011) also performed research at CATIE and, as I found, observed that coffee yield was significantly higher in FS treatments compared to shaded coffee treatments, however only in select years. These select years corresponded to a well-defined biennial trend in productivity, as others have observed (e.g. Cannell, 1985; DaMatta, 2004; DaMatta et al, 2007; van Oijen et al, 2010a), which may result in inconsistent trends between FS yield and agroforestry yield data in the literature. Furthermore, yield measurements for coffee are inconsistently calculated in the

literature. For example, in Haggar et al (2011), yield was estimated by the weight of the harvested coffee cherries over 6 consecutive years, similar to Campanha et al (2004) and Santos et al (2012), who used a similar method for 2 consecutive years. Soto-Pinto et al (2000) estimated yield through a combination of counting nodes and cherries on each branch, and weighing a subsample of cherries. In this study, following Meylan (2012), yield was estimated via the counting of total plant nodes and a subsample of cherry presence at one time. The yield estimates I calculated are within range of published yield data for coffee, including for some of my specific study sites (Campanha et al, 2004; Meylan, 2012). Therefore, although absolute yield data may be less reliable and inconsistent across the growing season, FN and cherry count data from my study are comparable across treatments and should be considered as an overall plant yield potential estimation.

5.4 Coffee agroforestry across climato-edaphic conditions

It has often been concluded that shading is not beneficial in optimal coffee growing conditions, and that it is only in suboptimal conditions that shade trees will promote productivity more so than full sun coffee (DaMatta et al, 2002). Due to the impact of altitude on climate, it is important in determining optimal coffee growing conditions. Differences at the three sites in this study consist of: i) altitude range, ii) *E. poeppigiana* management practices (pruning), and iii) fertilization rates. Although it is difficult to distill which one of these factors is driving differences in coffee response under FS and shade at the three sites, my data show strong differences between the sites and within sites across shade to FS coffee.

As expected, the productivity traits in this study increased as altitude increased, being statistically greater at the high altitude site compared to the low altitude site. This trend may be a reflection of the more optimal climate conditions experienced at higher altitudes (DaMatta, 2004). It may also reflect the greater amount of nutrient inputs applied at each study site, which, by chance, corresponds to the altitude gradient (see Chapter 3). Across the climato-edaphic gradient, the range of available soil N was 12 mg kg^{-1} to 690 mg kg^{-1}, which may be reflective of the large amount of variability in nutrient management regimes at the study sites. However, the available soil N data was determined via static point sampling, and so is limited in describing actual available soil N conditions throughout the year, as it cannot capture changes in response to climatic variability. Nevertheless, this data can serve as a proxy for available soil N conditions.

In the sampled sites, FN increased with increasing soil nitrates and ammonium. As described in Chapter 3, FN estimates were derived from total plant nodes and subsampled fruiting nodes. In the coffee literature, it has been observed that total plant nodes increase with both mulching and N fertilization (Cannell, 1973). This positive relationship between FN and available soil N is likely a reflection of the important role N plays in photosynthetic rates (Reis et al, 2009), as previously described. Since I found that A_{mass} increases with increasing available soil N, coffee plants growing in soils with high N availability are likely using the increased carbohydrates produced via photosynthesis towards the formation of productivity traits. In contrast, coffee plants growing in soils with low N availability with subsequently lower A_{mass}, likely require the limited carbohydrates produced for vegetative growth, reflecting the trade-off between productive and vegetative growth demands (DaMatta, 2004). However, since there were no observed significant differences in FN between the FS and shade treatments at each respective site, the shade trees did not exhibit competition for aboveground light or belowground nutrient resources and were, therefore, as productive as FS coffee.

Soil moisture had a positive correlation with G_s and A_{sat} in the sampled coffee plants. The positive correlation of G_s with soil moisture is reflective of the preference of stomata for moister air (DaMatta et al, 2002). When the air is drier, stomata tend to close to preserve leaf water content and promote leaf water use efficiency. Therefore, as the soil becomes moister, stomatal conductance is not restricted. When G_s is not limiting A_{sat}, there is an increase in A_{sat} with increasing soil moisture, which can be affected by climatic changes related to altitude, such as precipitation. For example, Franck and Vaast (2009) observed average daily photosynthetic values of approximately 6 μmol CO_2 m^{-2} s^{-1} in various light treatments in their study conducted in the Orosi Valley, Costa Rica at 1108 m.a.s.l., with an average annual rainfall of approximately 3000 mm for the area. When compared to studies conducted in Viçosa, Brazil at 650-690 m.a.s.l., daily averages for photosynthetic rates were approximately 2 and 3 μmol CO_2 m^{-2} s^{-1}, with an average annual rainfall of approximately 1200 mm (Araújo et al, 2008 and Chaves et al, 2008, respectively). Similar differences were found in G_s values in these studies. These findings from the published literature mirror my findings, in that coffee physiological traits can change given variations in altitude and the inherent changes in climato-edaphic conditions, highlighting potential underlying mechanisms for trait variability across sites.

When photosynthetic rates are compared across the three sites, rates were highest at the mid-altitude site in both treatments, suggesting that A_{sat} was not limited here. Between treatments, FS was consistently higher than shade, though the difference between treatments at the mid-altitude site (4%) was smaller than at the low altitude site (18%), and smaller still compared to the high altitude site (50%). At the high altitude site, the presence of shade had a negative effect on the rates of A_{sat} and G_s, being statistically lower in the shade treatment compared to the FS treatment. This reflects the understanding that shade tree presence in optimal coffee growing conditions, in this case at the high altitude site, may have a negative effect on coffee plant performance; whereas in suboptimal locations, such as the low and mid-altitude sites, shade tree presence can ameliorate coffee plant performance (Beer et al, 1998; DaMatta et al, 2002). This highlights the necessity to consider the benefits or drawbacks of shade tree presence on coffee performance over the numerous ecosystem services provided by shade trees across climato-edaphic conditions.

Across the climato-edaphic gradient, shade tree presence increased various leaf morphological traits, including leaf size, leaf dry mass, and LNC, although no significant differences were found. Similar to the shade tree biodiversity gradient, an increase in these resource acquiring traits was likely a reflection of the influence of incoming light, as well as the improved soil moisture and fertility that is characteristic of shaded treatments (Beer, 1987). As observed in natural plant communities (e.g. Richardson et al, 2013) and managed coffee systems (e.g. DaMatta, 2004), intraspecific plant morphological traits vary with environmental conditions. When comparing shade treatments at each site, leaf size and leaf dry mass were both highest in mid-altitude shade. This may be the result of coffee plants allocating resources to vegetative growth to maximize the efficiency of resource acquisition due to the denser and more uniform shade at this site, due to the lack of pruning of the *E. poeppigiana* shade trees (Vaast et al, 2008) on this specific coffee farm. Across the three sites, there was a positive correlation between LNC and SLA in the shade treatments, likely a reflection of the resource-acquiring nature of the coffee plant in shaded systems. As previously described, shaded plants have increased leaf size and LNC to enhance photosynthesis (e.g. Givnish, 1988; DaMatta et al, 2007). As altitude increased, the slope of the relationship decreased, likely because the site became more optimal for coffee growth, so the effects of shade tree presence decreased (Beer et al, 1998; DaMatta et al, 2002). Therefore the necessity of these resource-acquiring traits diminished with improving climato-

edaphic conditions. This supports the hypothesis observed in natural plant communities that intraspecific plant traits are correlated due to similar responses to abiotic conditions (Richardson et al, 2013). This trend was not found in the FS treatments, where insignificant relationships between LNC and SLA existed, likely because irradiance was not limiting and so these resource-acquiring traits were not necessary for adequate carbohydrate production. This intraspecific variability related to environmental conditions and management regimes highlights the importance of comparing climato-edaphic data and management protocols in future research across multiple geographical locations.

Chapter 6
Conclusion

While it has been shown that coffee-shade agroforestry systems require fewer inputs and achieve greater coffee yield stability, little work has focused on the shade tree and environmental processes that control intraspecific leaf trait variation of coffee crops. In this study, I analysed coffee plant response to a i) local scale gradient (light and nutrients) induced by shade tree diversity and ii) large scale gradient (climato-edaphic) induced by altitude. I originally hypothesized that coffee plant traits will display plasticity across such gradients, as variability of plant traits has been observed both in natural plant systems and managed systems. My results demonstrate that indeed, coffee plant traits are plastic in managed agroforestry systems across management regimes (shade tree species richness) and climato-edaphic conditions (altitude). Depending on the environmental pressures on the coffee plants, their plasticity capabilities allowed for shifts between resource acquiring and resource conserving traits, in order to maximize the efficient use of available resources. This plasticity enables coffee plants to acclimate to variable environments. However, this acclimation does not enable coffee plants to endure harsh climates, as plant stress still occurs.

The shift between resource acquiring and resource conserving traits was also observed at the local scale along the shade tree biodiversity gradient. As hypothesized, shade tree presence did not inhibit physiological or morphological traits, as some morphological traits increase in shaded conditions. However, shade tree presence did inhibit estimated coffee yield potential. The observed intraspecific variability in trait correlations was related to measured environmental conditions, as a result of interspecific interactions both above and belowground. This variability was due to biotic and abiotic interactions within managed sites, which highlights an important consideration for management. I found that coffee grown beneath two shade trees demonstrated enhanced physiological and morphological traits, suggesting a potential optimal shade tree biodiversity configuration at the studied altitude and climato-edaphic conditions. However, as observed in this study and cautioned in previous literature in both natural and managed systems, climatic and edaphic conditions must always be considered, due to the variable responses of functional plant traits.

Variations in environmental conditions were captured across the climato-edaphic gradient. It was observed that at low altitudes, coffee plants performed less than at higher altitudes, as hypothesized. Furthermore, shade tree presence moderated these effects at lower altitudes, more so than at high altitudes, representative of the benefits of shade trees in suboptimal conditions, corresponding with previous literature. However, in all of my research sites, full sun coffee had greater productivity potential. As discussed in previous literature, the decision to include shade trees is often reliant upon the farmer's desired outcome. Coffee plant longevity, and therefore, prolonged coffee plant productivity and performance, is enhanced by shade tree presence. In addition, shade trees can provide numerous ecosystem services to the coffee system, including reduced soil erosion, reduced nutrient leaching, and improved soil water retention. Shaded coffee provides a viable option for coffee farmers concerned about limiting environmental degradation related to farming practices, and mitigating climate change effects on coffee growth and productivity.

As observed in this study, interspecific plant interactions and intraspecific plant response vary dynamically across multiple gradients. This variability highlights current limitations in the data that have been deemed to be too site-specific to be applied to a wide range of scenarios. In order to overcome this limitation, there is a need to expand research into multi-factorial studies that incorporate analyses of abiotic conditions. My research addresses this limitation in the data and further supports the need for additional studies of a similar design. Future research should analyse intraspecific plant response along larger gradients of abiotic variables due to the observed variability in both functional plant trait means and trait correlations. My findings contribute to the growing literature on the underlying mechanisms that influence functional leaf trait plasticity of coffee and variability in intraspecific coffee plant response to changing biotic and abiotic conditions in a managed agroforestry system at the local (within farm) and regional (across multiple farms) scales.

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Appendix 1

Pearson's correlation coefficients between leaf traits across sampled coffee plants from all research sites and treatments. (*P<0.10; **P<0.05; ***P<0.01). Significant relationships are in bold.

	log FN	log Cherries	Above-ground Biomass	log Leaf Size	log Leaf Dry Mass	SLA	log SLM	log LDMC	Leaf Thickness
log FN	1.00								
log Cherries	**0.94*****	1.00							
Aboveground Biomass	0.03	0.04	1.00						
log Leaf Size	**-0.18***	**-0.17***	0.04	1.00					
log Leaf Dry Mass	**-0.25*****	**-0.18***	0.06	**0.89*****	1.00				
SLA	0.03	-0.05	-0.05	0.11	**-0.27*****	1.00			
log SLM	0.00	0.07	0.02	-0.13	**0.23*****	**-0.99*****	1.00		
log LDMC	-0.08	0.02	-0.09	-0.12	0.10	**-0.60*****	**0.55*****	1.00	
Leaf Thickness	0.15	0.13	0.10	0.09	**0.22*****	**-0.34*****	**0.32*****	0.00	1.00
Asat	-0.03	0.00	0.00	0.15	**0.22*****	-0.10	**0.14***	**-0.17****	**0.25*****
log Amass	-0.01	-0.06	0.02	**0.21*****	0.10	**0.19****	-0.13	**-0.38*****	**0.15***
log Gs	0.13	0.14	0.03	0.03	**0.14***	**-0.19****	**0.22*****	-0.10	**0.38*****
Rd	**0.21****	0.15	0.08	-0.05	-0.04	-0.03	-0.02	**-0.19****	0.02
LNC	-0.01	-0.06	0.09	**0.19****	0.05	**0.38*****	**-0.34*****	**-0.42*****	**-0.18****
log LNContent	**-0.24****	**-0.19****	0.08	**0.90*****	**0.95*****	-0.13	0.10	-0.04	**0.15***
PNUE	0.02	-0.01	-0.03	**0.15***	0.07	0.11	-0.07	**-0.28*****	**0.20****

Continued. Pearson's correlation coefficients between leaf traits across sampled coffee plants from all research sites and treatments. (*P<0.10; **P<0.05; ***P<0.01). Significant relationships are in bold.

	Asat	log Amass	log Gs	Rd	LNC	log LNContent	PNUE
Asat	1.00						
log Amass	**0.89*****	1.00					
log Gs	**0.72*****	**0.64*****	1.00				
Rd	-0.03	0.05	0.04	1.00			
LNC	0.07	**0.20****	**-0.24*****	0.09	1.00		
log LNContent	**0.23*****	**0.16****	0.06	-0.01	**0.36*****	1.00	
PNUE	**0.87*****	**0.93*****	**0.70*****	0.01	-0.11	0.03	1.00

Appendix 2

Spearman's correlation coefficients for leaf traits and environmental variables across sampled coffee plants from all research sites and treatments. (*P<0.10; **P<0.05; ***P<0.01). Significant relationships are in bold.

	FN	Cherries	Above-ground Biomass	Leaf size	Leaf Dry Mass	SLA	SLM	LDMC	Leaf Thickness
Total light transmittance	**0.47***	**0.52***	0.11	**-0.27***	**-0.38***	-0.03	0.04	-0.04	0.10
Soil moisture	0.13	0.08	**0.33***	0.10	**0.17**	-0.05	0.05	**-0.39***	0.10
Available soil N	**0.17***	0.05	**0.35***	0.05	0.04	0.06	-0.07	**-0.41***	0.08
Available soil P	**0.30***	**0.20**	**0.39***	-0.12	-0.03	0.01	-0.03	**-0.39***	0.13
Total soil N	**0.17***	0.15	**0.35***	-0.11	0.01	0.00	-0.02	**-0.30***	0.08
Total soil C	**0.26***	**0.21**	**0.27***	**-0.28***	-0.12	-0.08	0.07	**-0.17**	**0.23***

	Asat	Amass	Gs	Rd	LNC	LNContent	PNUE
Total light transmittance	0.04	0.05	**0.20**	**0.19**	**-0.14***	**-0.40***	0.10
Soil moisture	**0.29***	**0.26***	**0.20**	**0.30***	**0.38***	**0.27***	**0.14***
Available soil N	0.13	**0.15***	0.03	**0.31***	**0.31***	**0.14***	0.05
Available soil P	**0.15***	**0.16**	0.13	**0.40***	**0.25***	0.05	0.10
Total soil N	**0.25***	**0.27***	**0.28***	**0.32***	**0.19**	0.08	**0.21**
Total soil C	**0.14***	0.05	**0.20**	**0.26***	**-0.23***	**-0.21**	**0.15***