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Effects of Fertilizer and Shade Management on Nitrogen Mineralization, Nitrifying Microbial Abundance and Nitrogen-Fixing Capacity of Erythrina poeppigiana in Coffee (Coffea arabica) Agroforestry Systems in Costa Rica

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EFFECTS OF FERTILIZER AND SHADE MANAGEMENT ON NITROGEN MINERALIZATION, NITRIFYING MICROBIAL ABUNDANCE AND NITROGEN-FIXING CAPACITY OF *ERYTHRINA POEPPIGIANA* IN COFFEE (*COFFEA ARABICA*) AGROFORESTRY SYSTEMS IN COSTA RICA

by

STUART BARKER

(Under the Direction of Subhrajit Saha)

ABSTRACT

Worldwide chemical fertilizer use has increased by four times during the last 50 years. Conventional agricultural systems have a high nitrifying nature, resulting in a loss of nearly 70% of overall nitrogen (N) fertilizer inputs, an estimated economic loss of $81 billion. Over application of fertilizer is rampant in tropical developing nations in Central America, where coffee is major crop. Agroforestry offers ecologically sustainable land management strategies that promote the provision of ecosystem services such as, protection of biodiversity, climate change mitigation, and water and soil regulation. When legume trees are incorporated as the shade tree in coffee production, direct inputs of nitrogen can occur. The specific objectives of this study were, (1) to quantify the effects of inorganic fertilizer and shade treatments on soil organic carbon (SOC), (2) measure and quantify the mineralization rates under inorganic fertilizer and shade treatments, (3) determine if the spatial abundance of ammonia oxidizing bacteria (AOB) is affected by varying shade management, or by inorganic fertilizer treatment, and 4) measure the effects of inorganic fertilizer application on the transfer of biologically fixed N by *Erythrina poeppigiana* to *Coffea arabica* in agroforestry systems in the region. The field study was conducted in Aquiares, Costa Rica. Chemical fertilizer was applied between four treatments at the rate of 0, 110, 170, and 230 kg N ha^{-1} yr^{-1} and coupled with three shade treatments (no shade, managed shade, full shade) defined by the management strategies of the legume tree *E. poeppigiana.* Analyses showed no significant difference in total SOC by fertilizer and shade treatments. Measured NH_4^+ µmol/L NH_4^+ and NO_2^- + NO_3^- µmol/L NO_3^- concentrations differed significantly by the shade treatment, but net nitrogen mineralization rates were not significantly different by fertilizer or shade treatment. A significant difference in dsDNA copy number of AOB per soil g^{-1} was determined by shade treatment. Finally, fertilizer treatment demonstrated a significant effect on the potential for biologically fixed nitrogen from *E. poeppigiana* to be transferred to coffee planted in association. Coffee agroforestry systems with full shade *E. poeppigiana* legume trees offered additional inputs of nitrogen to mitigate the use of chemical fertilizers.

INDEX WORDS: Agroforestry, Nitrogen dynamics, Nitrifying bacteria, Nitrogen mineralization, Fertilizer, Coffee production

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CHAPTER 1

INTRODUCTION

With a projected 35% population increase by 2050, the globe faces a rise in demand for major crop production, challenged by climate change and food insecurity (Oyebamiji *et al.*, 2015). Population growth is expected to plateau around 9 billion people, however, a major correlate of this deceleration in growth is increased wealth and purchasing power (Godfray *et al.*, 2010). Increased population growth, coupled with higher purchasing power, leads to a greater demand for agricultural crops and commodities (Godfray *et al.*, 2010). Overall crop production is predicted to increase by 50% to meet the anticipated demand over the next few decades (Doos, 2002). If conventional agricultural technologies and practices are used to meet the anticipated demand, further environmental degradation is inevitable (Tilman *et al.*, 2002).

While working to increase crop production, we need to significantly decrease the climate impact of crop production (Smith *et al.*, 2008). Agricultural practices release significant levels of CO_2, CH_4, and N_2O into the atmosphere. While considering direct and indirect emissions, agriculture contributes about 17-32% of the anthropogenic greenhouse gas (GHG) emissions that drive climate change (Smith and Gregory, 2013). Direct emissions of agriculture contribute between 5.1 and 6.1 Gt CO_2-eq, 10-12% of global GHG emissions, mainly in the form of CH_4 3.3 Gt CO_2-eq yr^{-1} and N_2O 2.8 Gt CO_2-eq yr^{-1} (Smith and Gregory, 2013). However, the clearing of land and native vegetation for agricultural use releases large quantities of ecosystem carbon, such as CO_2 at 5.9 Gt CO_2-eq yr^{-1} (sd 2.9) (Smith and Gregory, 2013). The production and use of chemical fertilizers is another important source of GHG emissions (Ingram *et al.*, 2008).

The production of chemical fertilizers is energy intensive and contributes 0.3 to 0.6 Gt CO_2-eq/yr, roughly 0.6 to 1.2% of the world's total GHG (Smith and Gregory, 2013). The largest source of GHG emissions from the use of chemical fertilizers is during their production, which emits CO_2 with current production methods (Tallaksen *et al.*, 2015).

Climate – Agriculture:

CO_2 levels, temperature, and changing precipitation patterns will affect crop production (Parry *et al.*, 2004). In particular, tropical and sub-tropical regions and associated developing countries are predicted to have deleterious impacts on agriculture in the wake of climate change (Ingram *et al.*, 2008). While working to reduce the climate impact of crop production, it is important to consider the value of protecting freshwater resources, protecting biodiversity, and reducing the impact of food production on an array of ecosystem services. The ecosystem services framework has become a widely integrated framework to study the relationship between ecosystems and people (Fagerholm *et al.*, 2016). The framework describes how ecosystems provide a variety of important benefits to human well-being and can influence decisions made towards mitigating ecosystem degradation (Fagerholm *et al.*, 2016). The loss of ecosystem services that forests and natural ecosystems provide is attributed to the expansion of conventional agriculture (Wood *et al.*, 2016). Biodiversity determines the functioning and properties of ecosystems and their ability to generate goods and ecosystem services (Hooper *et al.*, 2005, Loreau *et al.*, 2001). Diversifying agricultural ecosystems has been advocated to improve agricultural resiliency and sustainability (Allinne *et al.*, 2016). Biodiversity sustains key ecological services, which can improve the ability of

agricultural ecosystems to internally maintain soil fertility, crop protection, and productivity (Altieri, 1999).

Agroforestry:

Agroforestry is a form of multiple cropping where two plant species exist that interact biologically, one of which is a woody perennial, and one of the species is managed for forage, annual or perennial crop production (Somarriba *et al.*, 2001). Agroforestry offers ecologically sustainable land management strategies that promote the provision of ecosystem services (De Beenhouwer *et al.*, 2013). Agroforestry is widely adopted in the world's tropical and subtropical regions, and combines the provision of agricultural and forestry products with outputs such as, climate change mitigation, and water and soil regulation (Asase and Tetteh, 2016, Fagerholm *et al.*, 2016). Agroforestry systems mimic natural ecosystems, promote carbon and nutrient cycles and improve sustainability in humid tropical agriculture (Munroe *et al.*, 2015). Tropical forests are among the most biodiverse ecosystems on Earth, providing essential ecosystem services to the benefit of society (Wright, 2005, Gardner *et al.*, 2009). In the past century, tropical forests have been subjected to dramatic changes through anthropogenic land conversion, largely resulting from a shift to agricultural land (Lambin *et al.*, 2003). From 2010 to 2015, tropical forest area declined at a rate of 5.5 M ha y^{-1} (Keenan *et al.*, 2015). Tropical agroforestry systems have been proposed as a way to sustain biodiversity and the associated ecosystem services such as, buffering of climatic extremes and enhancing soil productivity (De Beenhouwer *et al.*, 2013). In an analysis of biodiversity and ecosystem services benefits in coffee agroforestry, De Beenhour (2013) reported response ratios of forest species richness and total species richness were significantly

lower in the more intensively managed than in more natural land use categories; with a decline of -46% when comparing agroforest with plantation, and -11% when comparing forest with agroforest.

Coffee:

Coffee (*Coffea arabica*) is one of the most valuable agricultural exports from developing nations, employing over 25 million people worldwide, and is cultivated in some of the world's most biodiverse regions (Ricketts *et al.*, 2004). Full sun, monoculture, coffee production produces high economic yields using dwarf-type high yielding varieties, with high planting densities (Lopez-Rodriguez *et al.*, 2015). Higher yields are attributed to increased photosynthetic expression of the coffee plant (Lopez-Rodriguez *et al.*, 2015). While full sun production can produce higher yields under optimal climatic conditions, it comes at the expense of high production cost, loss of biodiversity, increased nutrient run-off, and the reduction in the provision of ecosystem services. (Munroe *et al.*, 2015, Jha *et al.*, 2014). Incorporating shade trees in coffee production can provide an array of benefits. Shade trees can diminish crop exhaustion, increase longevity of coffee plants, improve soil fertility, and provide timber value, along with the provision of fruits and other products (Beer *et al.*, 1997).

In an effort to improve their gross national income, many Latin American countries underwent a technological transformation of traditional agroecosystems in order to maximize coffee yields, production and profits for an export market (Castro-Tanzi *et al.*, 2014). In Costa Rica, ranked in the top 15 coffee producing countries between 2000 and 2009, this transformation resulted in a near elimination of the shade tree canopy, increased planting densities of high-yielding varieties, and increased use of

agrochemicals, without proper consideration and evaluation of the ecological consequences (Castro-Tanzi *et al.*, 2014). Intensively managed coffee (*Coffea arabica*) systems in Costa Rica, grown under heavily pruned leguminous trees or in unshaded monocultures that receive high fertilization rates (~250 kg N ha^{-1} yr^{-1}), are a suspected cause of the increased ground water nitrate (NO_3^-) concentration in the Central Valley (Harmand *et al.*, 2007). Additionally, intensive fertilizer use can lead to an increase in exchangeable acidity, and a decrease in cation exchange capacity (Barak *et al.*, 1997), which can lead to yield reductions because of soil acidification overtime (Matsuyama *et al.*, 2005). Moreover, chemical fertilizer use in conventional coffee production can account for up to 55% of total variable costs for farmers (Lyngbaek *et al.*, 2001). Therefore, moderate reductions in chemical fertilizer use may provide a cost saving mechanism that can improve environmental performance without dramatic reductions to yield (Castro-Tanzi *et al.*, 2014).

Nitrogen dynamics:

Above and below-ground nitrogen (N) transfer from leguminous shade trees to the coffee crop is an important N source in agroforestry systems (Chesney, 2008, Isaac *et al.*, 2012, Munroe and Isaac, 2014). When legume trees are incorporated as the shade tree, direct inputs of N occur through mycorrhizal fungi networks or absorption of N-rich root exudates of the legume tree by the coffee plant (Chesney and Nygren, 2002), (Nygren and Leblanc, 2015). The amounts of N contributed will depend on the factors that limit N fixation, which include: limited nutrient supply; inappropriate symbionts; nodule effectiveness and activity; moisture supply; and presence of inhibitors, such as combined nitrogen (Munroe and Isaac, 2014). Castro-Tanzi *et al.* (2014) reported N

contributions of 24 ± 3.3% from biological N_2 fixation when coffee was grown in association with tree species of the genera *Erythrina*; however, with the facilitation of organic and inorganic forms of N being dependent on soil microbial communities, it is of interest to study the factors affecting spatial availability of usable N to crops (Hayatsu *et al.*, 2008).

The microbial process of nitrification is a key and integral part of the soil N cycle, which influences the fate of N in terrestrial systems (Ouyang *et al.*, 2016). Inorganic N forms such as, NH_4^+ or NO_3^-, are major forms of N uptake for plants in agricultural systems (Subbarao *et al.*, 2013). The biological oxidation of ammonia (NH_3) or ammonium (NH_4^+) to NO_3^- is mediated by ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and the nitrite-oxidizing bacteria (NOB) groups (Hayatsu *et al.*, 2008, Ouyang *et al.*, 2016). The first step of the nitrification process is the oxidation of NH_3 or NH_4^+ to hydroxylamine (H_3NO) and nitrite (NO_2^-), and this step is catalyzed by the AOA and AOB groups (Ouyang *et al.*, 2016). The last nitrification step is the oxidation of NO_2^- to NO_3^- and is performed by the NOB group (Wertz *et al.*, 2008). Some studies suggest AOB are more dominant over AOA in agricultural soils, but conflicting reports suggests the abundance and activity of AOA and AOB are affected by environmental conditions such as, ammonium (NH_4^+) concentration, N availability, temperature, salinity, moisture, pH, soil organic matter, and pH (Zhou *et al.*, 2016).

In agricultural systems, rapid nitrification rates result in inefficient N use by crops, leading to increased leaching and environmental pollution (Subbarao *et al.*, 2013). Natural ecosystems exploit various N forms (organic and inorganic) and utilize multiple pathways to regulate N flows, restricting N flow solely through the nitrification path

(Smolander *et al.*, 1998). By utilizing leguminous shade trees in coffee production, the total N pool can be supplemented by biologically fixed N and organic N forms from leaf litter and root turnover (Chesney and Nygren, 2002). Additionally, forms of ammonium based fertilizers could reduce the amount of leaching and N pollution over NO_3^-, as NH_4^+ is much less mobile in the soil than NO_3^- and, therefore, has a longer N retention time in the root zone of plants, increasing the time for uptake (Subbarao *et al.*, 2013).

In this study, I sought to evaluate the N supply and dynamics in coffee (*Coffea arabica*) agricultural systems under different management constraints, ranging from intensively managed no-shade monoculture production, to less intensively managed full-shade agroforestry systems. The study was conducted in the central-Caribbean region of Costa Rica, in an experimental designation within the Aquiares coffee farm, a Rainforest Alliance ™" certified farm, characterized by andisol soils (Taugourdeau *et al.*, 2014). The experiment was carried-out in a split-plot design. Urea-based fertilizers were applied between four treatments at the rate of 0, 110, 170, 230 kg N ha^{-1} yr^{-1}. Each fertilizer treatment plot contained three shade affects. The first being characterized by the absence of the legume tree *E. poeppigiana* (no shade monoculture, NS), the second having pruned *E. poeppigiana* (managed shade, MS), and the third having free-growing *E. poeppigiana* (full-shade, FS).

The specific objectives of this study were:

1) To quantify the effects of inorganic fertilizer and shade treatments on soil organic carbon (SOC).

2) To measure and quantify the mineralization rates under inorganic fertilizer and shade treatments.

3) To determine if the spatial abundance of AOB is affected by varying shade management, or by inorganic fertilizer treatment.

4) To measure the effects of inorganic fertilizer application on the transfer of biologically fixed N by *E. poeppigiana* to *Coffea arabica* in agroforestry systems in the region.

CHAPTER 2
MATERIALS AND METHODS

FIELD STUDY

Study location:

The study was conducted in the central-Caribbean country of Costa Rica, in one of the largest coffee farms in the country, the Aquiares farm (9˚ 56’ 19” N, 83˚ 43’ 46” W) (660 ha), “Rainforest Alliance ™” certified, located 15 km from CATIE (Centro Agronómico Tropical de Investigación y Enseñanza) (Taugourdeau *et al.*, 2014). The Aquiares farm is 1020 m above sea level on the slopes of the Turrialba Volcano, with soils belonging to the order of andisols (USDA, 1999). Andisols are soils that develop from volcanic material, with high organic matter and allophane content and high infiltrability (Benegas *et al.*, 2014, Taugourdeau *et al.*, 2014). The Köppen-Geiger climate classification ranks the climate of the area as tropical humid with no dry season (Peel *et al.*, 2007). The mean annual rainfall at the Aquiares farm between 1973–2009 was 3014 mm, with the driest month being March (123 mm), and the wettest month being December (329 mm) (Taugourdeau *et al.*, 2014). The Aquiares farm is planted with coffee (*Coffea arabica* L. var. *caturra*) with an initial planting density of 1.11 m on the row and 1.43 m in-between rows, with 2 coffee stumps per position, about 6300 positions ha^{-1}.

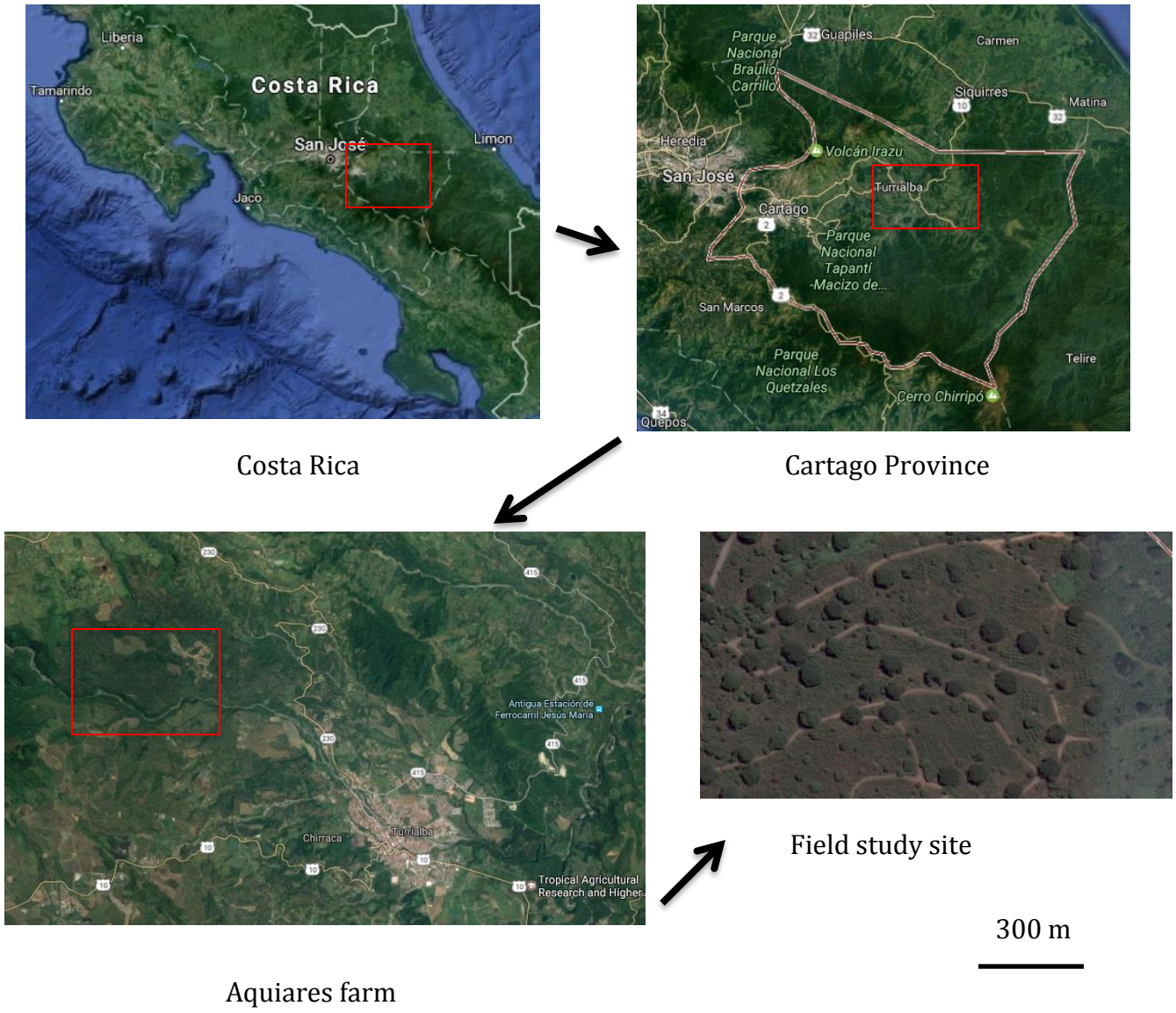

Figure 1. Map of Costa Rica including the province of Cartago, city of Turrialba and an aerial view of the field study site in the Aquiares farm (Google Maps, last accessed March 2017).

Site description and experimental design:

The experiment was conducted in a four-year old experimental agroforestry site located within the Aquiares farm. The experimental site is four hectares, which is divided into 12 experimental plots (~ 0.3 ha each) that differ by fertilizer and shade management

practices. The experimental site was fashioned in a split-plot design, where each whole plot received a fertilizer treatment and contained three subplot shade treatments within. Urea fertilizer was applied between four treatments at the rate of 0, 110, 170, and 230 kg N ha^{-1} yr^{-1}. The three shade treatments were defined by the management strategies of the legume tree *E. poeppigiana,* planted in association with the coffee crop. The shade treatments consisted of 1) no shade, full sun monoculture, were no *E. poeppigiana* is present; 2) managed shade, where *E. poeppigiana* is fully pruned twice per year; and 3) full shade, where *E. poeppigiana* is free growing. The average height of pruned *E. poeppigiana* was about 4–5 m, while average height of free-growth *E. poeppigiana* was 30–33 m.

Having three shade treatments split within four fertilizer treatments allowed for 12 treatment combinations, which were replicated 3 times, totaling 36 subplots. The four fertilizer treatments were distributed at random amongst the 12 plots.

Figure 2. Map of experimental site, includes fertilizer treatments by block (kg N $ha^{-1}y^{-1}$) and shade management within each block (no shade, managed shade, full shade). Boxes within each plot indicate subplots containing shade treatments. Circles indicate full growth *Erythrina* trees.

*Note: The full shade treatment in plot number 2 was excluded from all analyses. The full shade tree in subplot number 2 was struck by lightning and was removed.

Table 1. Fertilizer treatments were applied to the whole plot in a completely randomized design (kg N $ha^{-1}y^{-1}$)

Whole plot numbers	Fertilizer treatment
2, 6, 8	0
1, 4, 9	110
10, 11, 12	170
3, 5, 7	230

Statistical analyses:

All statistical analyses were completed using JMP 12 (Cary, NC). Analysis of variance under a split–plot design was used for data that fit a normal distribution for SOC Mg ha^{-1}, total NH_4^+ and NO_3^- µmol /L, N mineralization rates, and dsDNA copy number of AOB per soil g^{-1} under fertilizer treatments (0, 110, 170, and 230 kg N ha^{-1} y^{-1}) and shade treatments (no shade, managed shade, and full shade). Connecting letters reports were generated by Tukey's HSD. Analysis of mean $\delta^{15}N$ was carried out by analysis of covariance between distances (1, 2, 3, 4, 6 and 10 m) from managed *Erythrina* trees and fertilizer treatments (0 and 230 kg N ha^{-1} y^{-1}).

Total Soil Organic Carbon Analysis:

Two sets of four soil-cores were taken from each subplot to 20 cm in depth with a 0.5 L soil auger and combined to form a composite sample. Soil cores were taken from the center of each subplot to avoid edge effect from neighboring treatments. After collection, composite samples were dried in a drying oven for 72 hours at 60° C. Once dried the soils were sieved at 2 mm to remove gravels and plant material. To process the soil samples for total nitrogen (TN) and total carbon (TC) analysis, the size of the soil granules required further reduction. The soil samples were placed in scintillation vials with two ceramic beads and ground for 5 minutes using a SPEX Prep Mixer/Mill 8000k (Metuchen, NJ). A Thermo Scientific Flash 2000 TN/TC Soil Analyzer (Waltham, MA) was used to assess the samples for TC content. Total SOC was calculated by using the following formula (Eq. 1), calculating total SOC in Mg ha^{-1} (Saha et al., 2010):

Equation 1: C storage = C concentration × BD × Soil Depth × Fraction weight

Where:
C storage = Mass measured in Mg ha^{-1} (Mg = megagram)
C concentration = g per kg of soil contained in sample
BD = Bulk density (Mg m^{-3})
Depth = Depth of soil profile (cm)
Fraction weight = Percent of the fraction in the sample

N mineralization rates:

Composite soil cores from each subplot were used for total NH_4^+ µmol /L NH_4^+ and NO_2^- + NO_3^- µmol /L NO_3^- and net N mineralization rate analyses. Composite soil samples were taken (0–20 cm) directly from the field to the lab at CATIE for NO_3^- and NH_4^+ extractions. Once in the lab, 50 g of soil was weighed for each extraction. For each subplot, an initial and final subsample of soil was taken to calculate total NO_3^- and NH_4^+

concentration and net N mineralization rates over time. Initial subsamples underwent NO_3^- and NH_4^+ extraction immediately by 2M KCL and were stored until laboratory analysis. Final subsamples were sealed with parafilm and placed in an incubation chamber at 25°C for 14 days before undergoing NO_3^- and NH_4^+ extraction with 2M KCL. Liquid NO_3^- and NH_4^+ extraction samples were preserved in vacuum sealed sample tubes and shipped to CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Avenue Agropolis, 34398 Montpellier Cedex 5 France) for total NH_4^+ µmol /L NH_4^+ and NO_2^- + NO_3^- µmol /L NO_3^- calculation and analyses. Net N mineralization was calculated as the change in (NH_4^+ µmol /L NH_4^+) + (NO_2^- + NO_3^- µmol /L NO_3^-) during the 14 day incubation (Neill *et al.*, 1997).

Nitrifying Bacteria Analysis:

Composite soil sampling was used to quantify the microbial communities of the plots. Composite samples were taken in each subplot (n = 70) to 20 cm using a 0.5 L auger. Composite samples were taken within the same day and immediately taken to the lab space in CATIE and stored in the freezer at approximately -5°C for 10 hours. The following morning, samples were vacuum freeze-dried at -52°C for DNA preservation. Soil microbial DNA extractions were performed using a PowerSoil DNA Isolation kit (MO BIO, Carlsbad, CA, USA).

Quantitative polymerase chain reaction (qPCR) was used for absolute DNA quantification of the target AOB microbial group. The qPCR methods established by Jin *et al.*, 2010 were followed for target group quantification. qPCR was performed using the QuantStudio™ 6 Flex Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) with SYBR-Green I fluorescent dye. All PCR mixtures used recommended 20 µl

protocol for SYBR consisting of 10 µl of 2X PCR Master Mix, 0.4 µl of 10 µM forward and reverse primers, 5 µl of isolated DNA, and 4.2 µl of nuclease-free water. We utilized AOB target primer sets by Rotthauwe *et al.*, 1997; Table 2. The AOB amoA PCR program included 10 min at 95° C; 40 cycles of 30 s at 95° C, 1 min at 57° C, and 1 min at 72° C; and a final elongation step at 72° C for 10 seconds. Melting curve confirmation analysis was conducted for all reactions to ensure the correct target amplification. Standards for the AOB primer set were created from DNA isolated from soil (Moore *et al.*, 2016). Product bands were excised from agarose gels then extracted and purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The resulting fragments were ligated into TOPO vectors and cloned into TOP10 competent cells (Life Technologies, Carlsbad, CA) using manufacturer protocols. Plasmids were extracted from the resulting culture using QIAprep Spin Miniprep Kits (Qiagen, Hilden, Germany) and plasmid concentrations were quantified using a NanoDrop ND-1000 UVeVis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Ten-fold serial dilutions were used to create standard sets (ranging from 1.5×10^2 to 1.54×10^8 dsDNA copy number soil g^{-1}). Average standard curve efficiencies were 92.65% with an $r^2 > 0.98$. The following formula was used to absolute quantification of AOB:

$$\text{Number of copies (molecules)} = \frac{\text{X ng} * 6.0221 \times 10^{23} \text{molecules/mole}}{(\text{N} * 660 \text{g/mole}) * 1 \times 10^{9} n\text{g/g}}$$

Where:
X = amount of amplicon (ng)
N = length of dsDNA amplicon
660 g/mole = average mass of 1 bp dsDNA

Table 2. Primers used for qPCR amplification

Target gene	Primer	Sequence (5'-3')	Reference
*amo*A	amoA-1F	GGGGTTTCTACTGGTGGT	Rotthauwe et al., 1997
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC	Rotthauwe et al., 1997

N-Fixing Capacity:

Two stable isotopes of N exist, ^{14}N and ^{15}N. The heavy isotope, ^{15}N, occurs in atmospheric N_2 at a constant of 0.3663 atoms % (Dawson *et al.*, 2002). N_2 exists in a range of abundances within soil, typically at an enriched abundance (Munroe et al, 2014). If a sample is compared to an absolute abundance ratio derived from atmospheric N, researchers can obtain a delta (δ) ^{15}N value (Martinelli et al., 1999, Dawson et al., 2002). A sample enriched in ^{15}N relative to natural abundance would be an example of a positive $\delta^{15}N$ value; in contrast a negative $\delta^{15}N$ would be associated with lower ^{15}N (Dawson et al. 2002). When small $\delta^{15}N$ values are compared with a non-N_2-fixing reference plant with larger $\delta^{15}N$ values, enrichment of available soil N is taking place, and can be used to quantify the percentage of N derived from the atmosphere by N_2-fixing plants via the natural abundance method (Munroe et al., 2014).

The natural ^{15}N abundance method was used to calculate if there is variation in the N fixing capacity of *E. poeppigiana* by fertilizer treatment. Sample plots 2, 3, 5, 6, 7 and 8 were utilized to compare biological N_2 fixation by *E. poeppigiana*. Plots 2, 6, and 8 formed the control group with no inorganic fertilizer application and 3, 5, and 7 formed the treatment group with an application of 230 kg N $ha^{-1}y^{-1}$ of ammonia fertilizer.

To determine whether the N_2-fixing capacity of *E. poeppigiana* is affected by fertilizer application and at what distance from *E. poeppigiana* does *C. arabica* L. var.

caturra receive fixed N, composite leaf samples (n = 84) were taken. Two *E. poeppigiana* trees (n = 12) were selected from each plot and two transects along the planted *C. arabica* L. var. *caturra* rows were created, sampling at specified distances (1, 2, 3, 4, 6, 10 m) from the *E. poeppigiana* trees. Ten mature leaves from each *E. poeppigiana* trees were selected, dried for 48 hours at 60° C and ground to form a composite sample. Forty mature coffee leaves (identified with assistance from an experienced farm manager) were selected from each *C. arabica* L. var. *caturra* plant, dried for 48 hours at 60° C, and grinded to form a composite sample. Leaf samples were then sealed in centrifuge vials and transported to the SISSIL (Skidaway Institute Scientific Stable Isotope Laboratory, Savannah, GA, USA) for ^{15}N analyses.

CHAPTER 3

RESULTS

No significant difference for SOC Mg ha^{-1} was detected between treatments of fertilizer, shade, or shade crossed with fertilizer (p= 0.3765, 0.9878, 0.6464, Table 3).

Table 3. Analysis of variation of total SOC Mg ha^{-1} under fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}) and shade treatment (no shade, managed shade, and full shade). Soil Organic Carbon Mg ha^{-1}

Whole Plot	DF	Mean square	F Ratio	Prob. > F
Fertilizer Treatment	3	4697.78	1.1799	0.3765
Error	8	4024.3	14.3215	
Subplot	**DF**	**Mean square**	**F Ratio**	**Prob. > F**
Shade	2	3.4611	0.0123	0.9878
Shade Treatment *Fertilizer Treatment	6	199.747	0.7109	0.6464
Error	15	281		

*Indicates significance

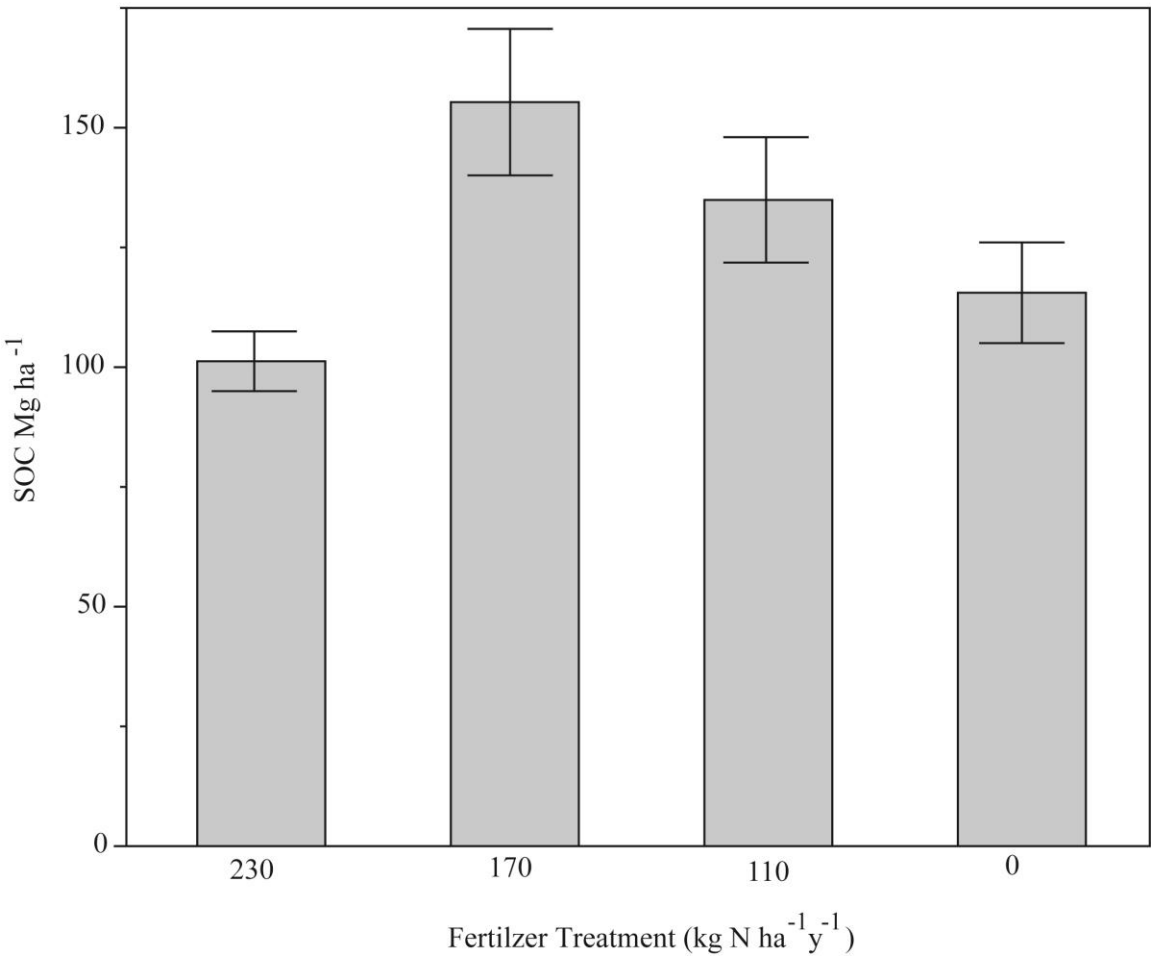

Figure 3. Total SOC Mg ha^{-1} by fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

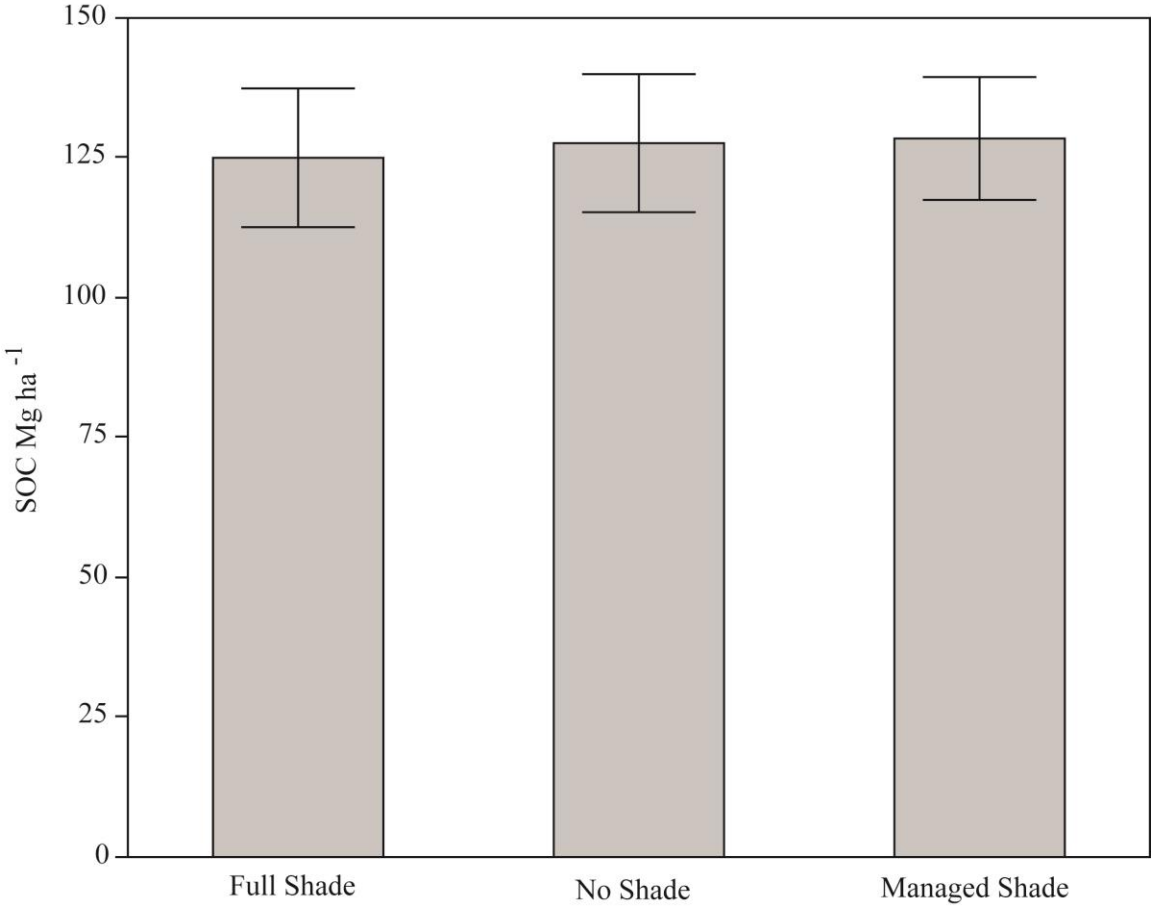

Figure 4. Total SOC Mg ha^{-1} by shade treatment (no shade, managed shade, and full shade). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

No significant difference for the fertilizer and shade crossed with fertilizer treatments were detected (p= 0.9455, 0.5467). A significant difference in mean NH_4^+ µmol/L NH_4^+ for the shade treatment was detected. (p= 0.0262, Table 4).

Table 4. Analysis of variance of NH4 µmol/L NH4 under fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}) and shade treatment (no shade, managed shade, and full shade).
NH_4^+ µmol/L NH_4^+

Whole Plot	**DF**	**Mean square**	**F Ratio**	**Prob>F**
Fertilizer Treatment	3	4.59611	0.1204	0.9455
Error	8	38.5651	9.1488	
Subplot	**DF**	**Mean square**	**F Ratio**	**Prob>F**
Shade	2	19.7611	4.6879	0.0262*
Shade Treatment *Fertilizer Treatment	6	3.61645	0.8579	0.5467
Error	15	4.2153	4.22	

*Indicates significance

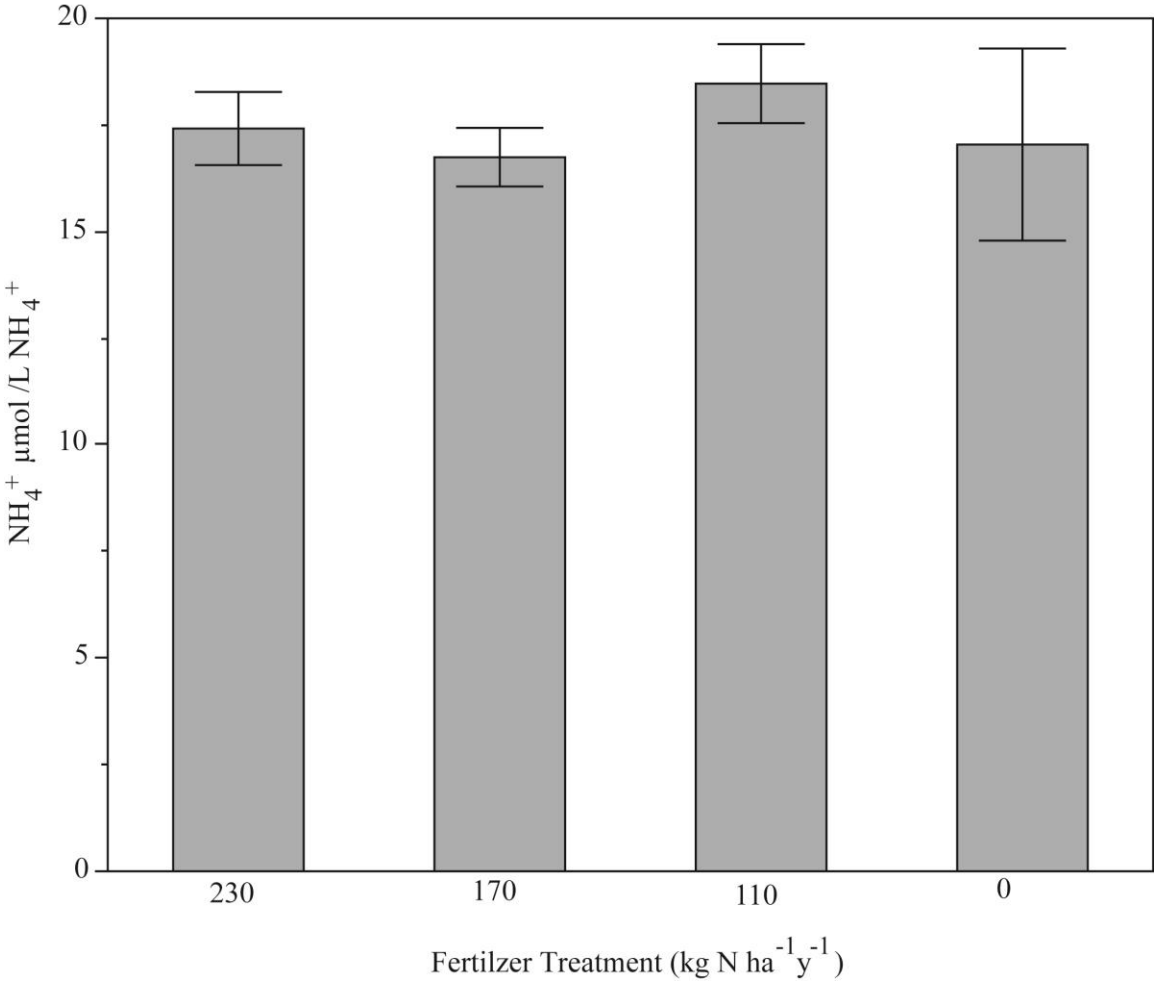

Figure 5. Total NH_4^+ µmol/L NH_4^+ by fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

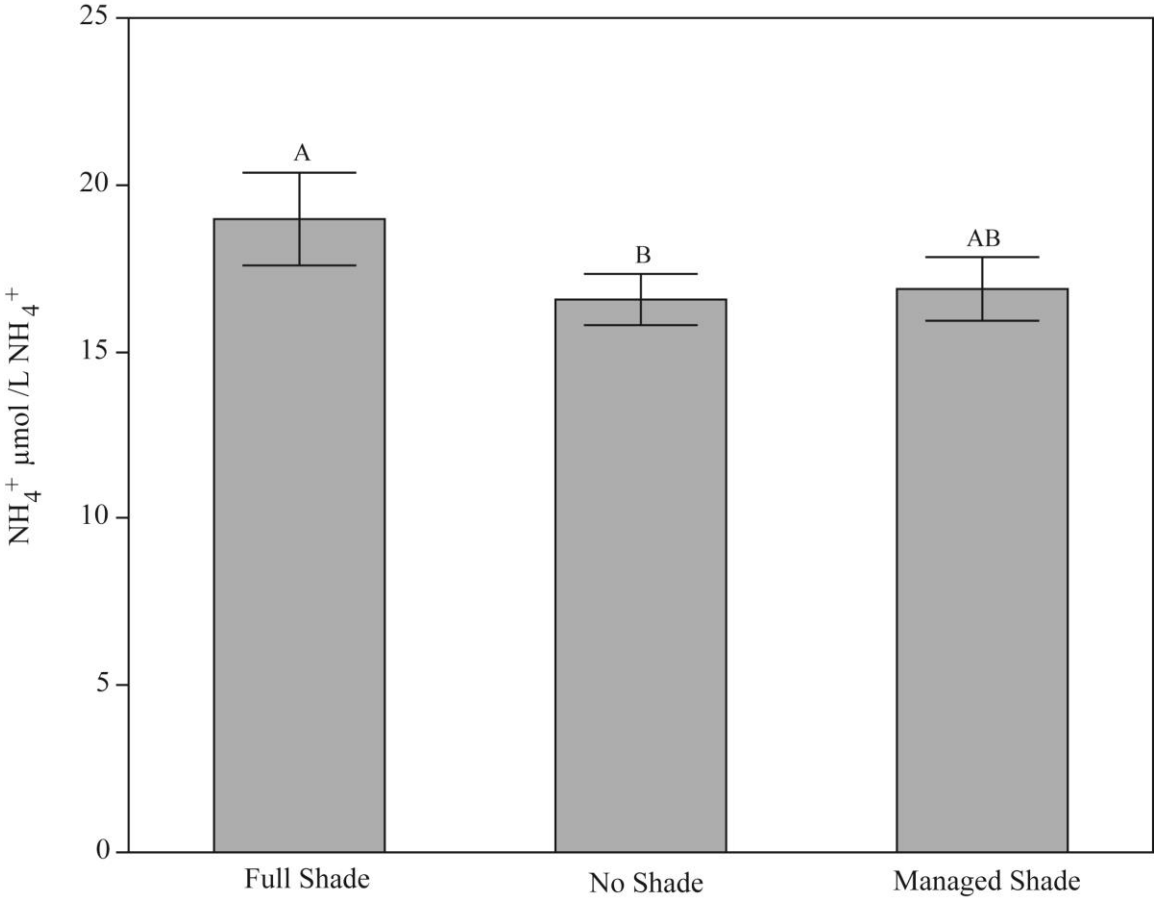

Figure 6. Total NH_4^+ µmol/L NH_4^+ by shade treatment (no shade, managed shade, and full shade). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

No significant difference for the fertilizer and shade crossed with fertilizer treatments were detected (p= 0.5472, 0.2496). A significant difference in mean NO_2^- +NO_3^- µmol/L NO_3^- for the shade treatment was detected. (p= 0.0003, Table 5).

Table 5. Analysis of variance of NO_2^-+NO_3^- µmol/L NO_3^- under fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}) and shade treatment (no shade, managed shade, and full shade).

NO_2^-+NO_3^- µmol/L NO_3^-

Whole Plot	**DF**	**Mean square**	**F Ratio**	**Prob>F**
Fertilizer Treatment	3	1769.23	0.7597	0.5472
Error	8	2345.2	2.5649	
Subplot	**DF**	**Mean square**	**F Ratio**	**Prob>F**
Shade	2	13396.5	14.6511	0.0003*
Shade Treatment *Fertilizer Treatment	6	1356.25	1.4833	0.2496
Error	15	914.36		

*Indicates significance

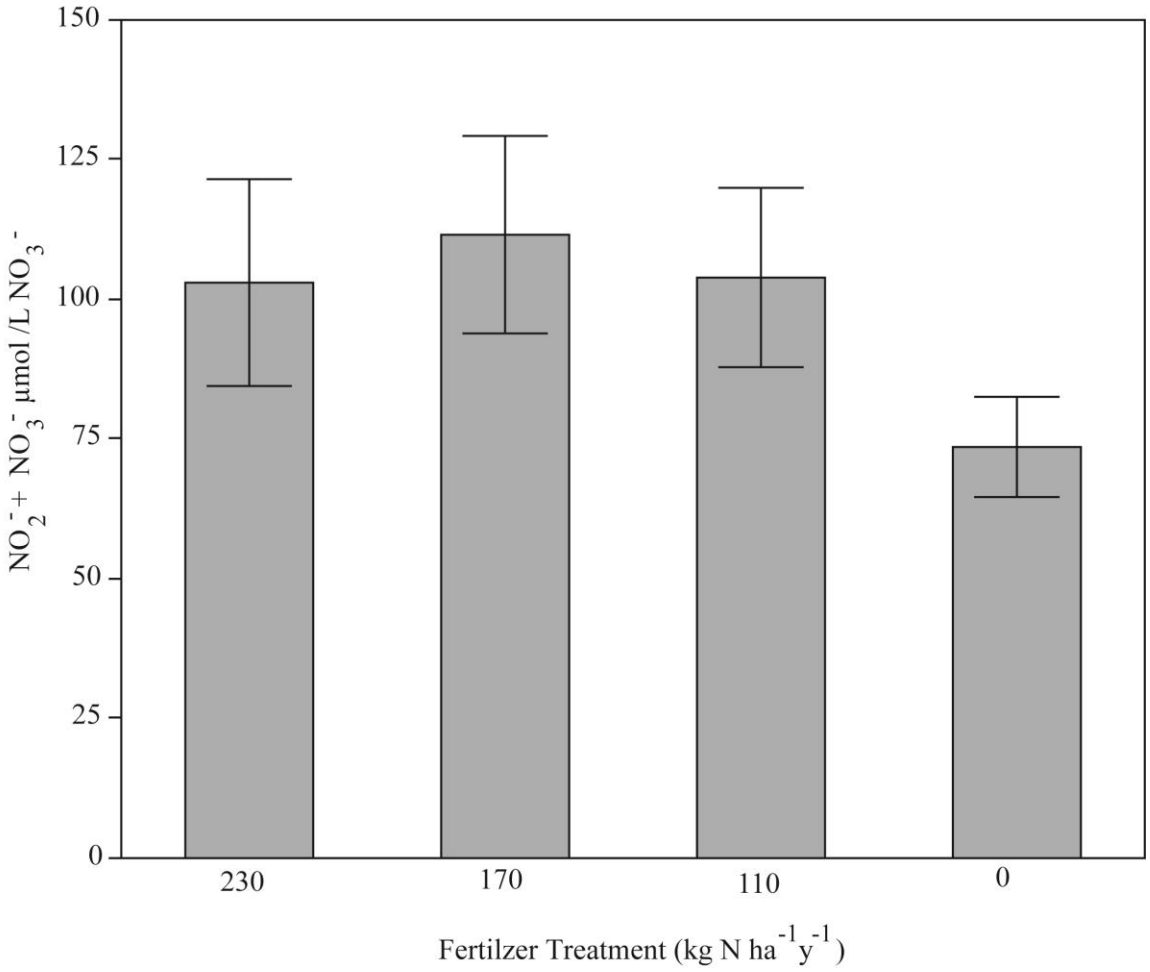

Figure 7. Total NO_2^-+NO_3^- µmol/L NO_3^- by fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

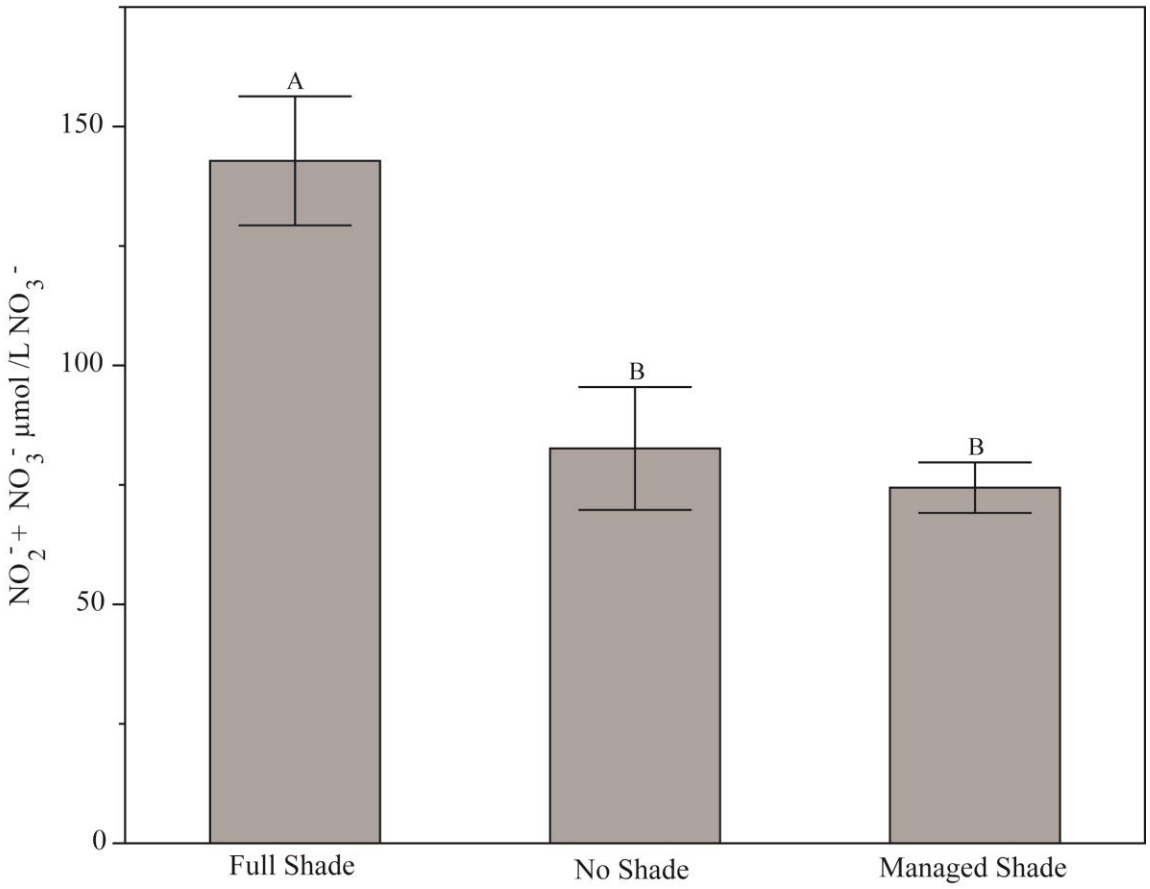

Figure 8. Total NO_2^-+NO_3^- µmol/L NO_3^- by shade treatment (no shade, managed shade, and full shade). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

No significant difference for net N mineralization rates was detected between treatments of fertilizer, shade, or shade crossed with fertilizer (p= 0.6658, 0.9612, 0.193, Table 6).

Table 6. Analysis of variance of net N mineralization rates under fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}) and shade treatment (no shade, managed shade, and full shade).

Net N Mineralization

Whole Plot	**DF**	**Mean square**	**F Ratio**	**Prob>F**
Fertilizer Treatment	3	6.05709	0.5438	0.6658
Error	8	11.2513	7.922	
Subplot	**DF**	**Mean square**	**F Ratio**	**Prob>F**
Shade	2	0.05634	0.0397	0.9612
Shade Treatment *Fertilizer Treatment	6	2.39173	1.684	0.193
Error	15	1.42027		

*Indicates significance

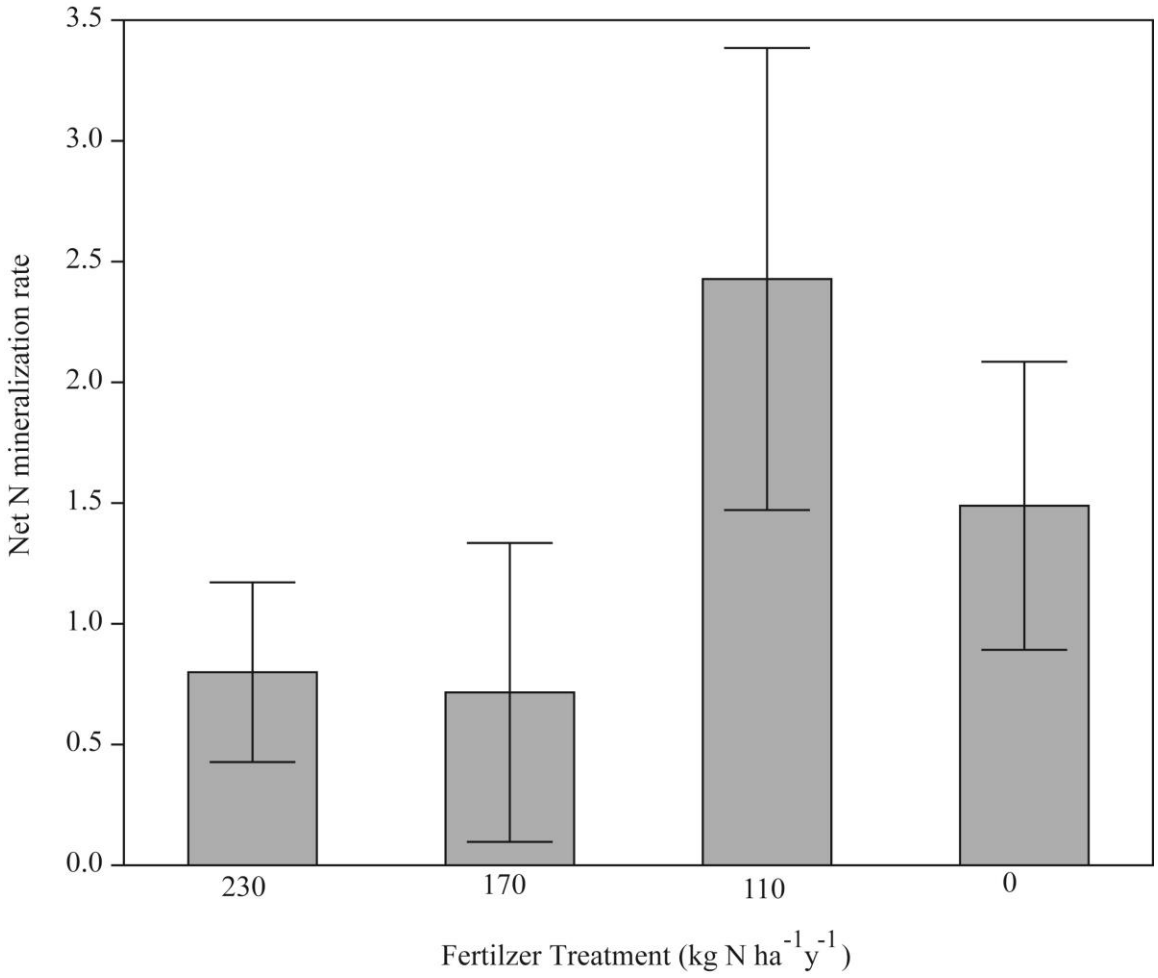

Figure 9. Net N mineralization rates by fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

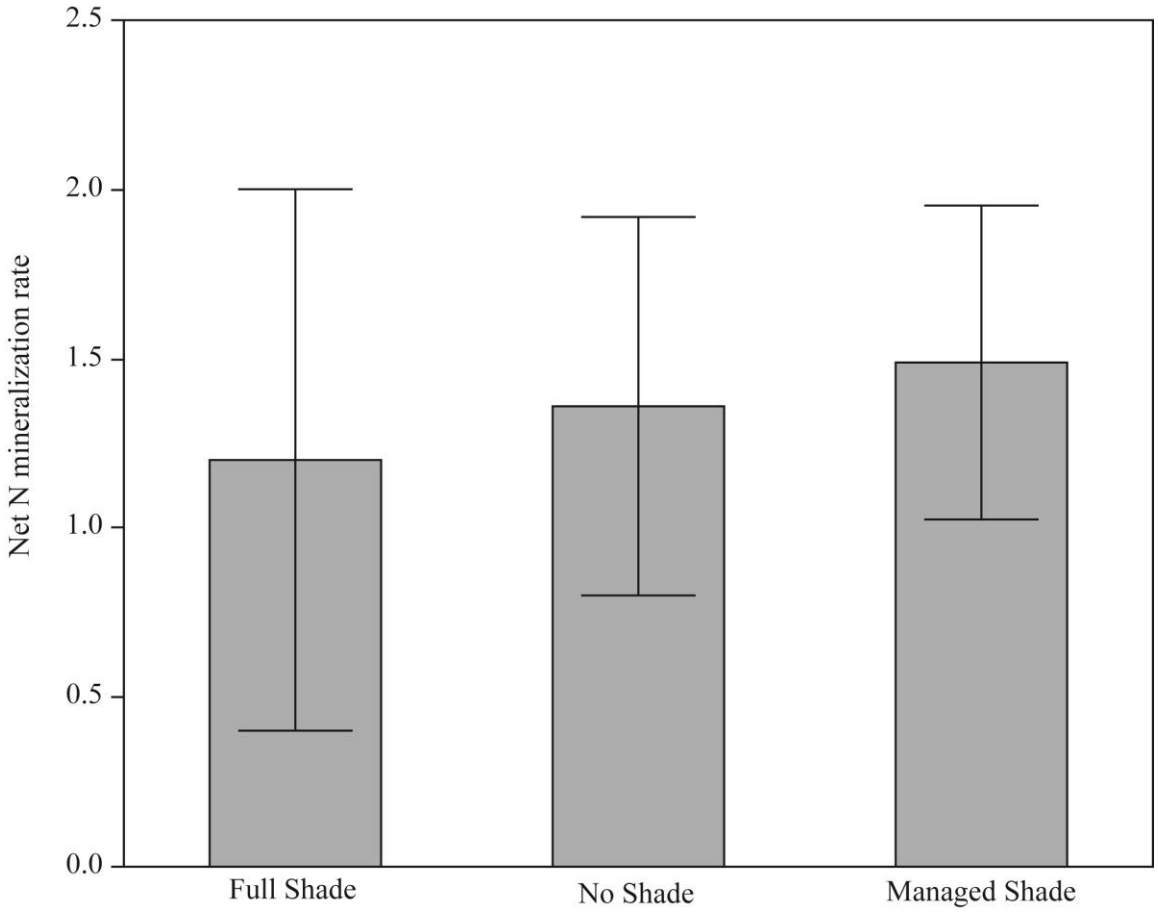

Figure 10. Net N mineralization rates by shade treatment (no shade, managed shade, and full shade). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

No significant difference in dsDNA copy number of AOB per soil g^{-1} for the fertilizer and shade crossed with fertilizer treatments were detected (p= 0.8345, 0.8971). A significant difference in dsDNA copy number of AOB per soil g^{-1} for the shade treatment was detected. (p= 0.0166, Table 7).

Table 7. Analysis of variance of dsDNA copy number of AOB per soil g^{-1} under fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}) and shade treatment (no shade, managed shade, and full shade).

dsDNA copy number of AOB per soil g^{-1}

Whole Plot	**DF**	**Mean square**	**F Ratio**	**Prob>F**
Fertilizer Treatment	3	7.66E+07	0.2858	0.8345
Error	8	2.67E+08		
Subplot	**DF**	**Mean square**	**F Ratio**	**Prob>F**
Shade	2	1.76E+09	5.4532	0.0166*
Shade Treatment *Fertilizer Treatment	6	1.14E+08	0.3531	0.8971
Error	15	3.22E+08		

*Indicates significance

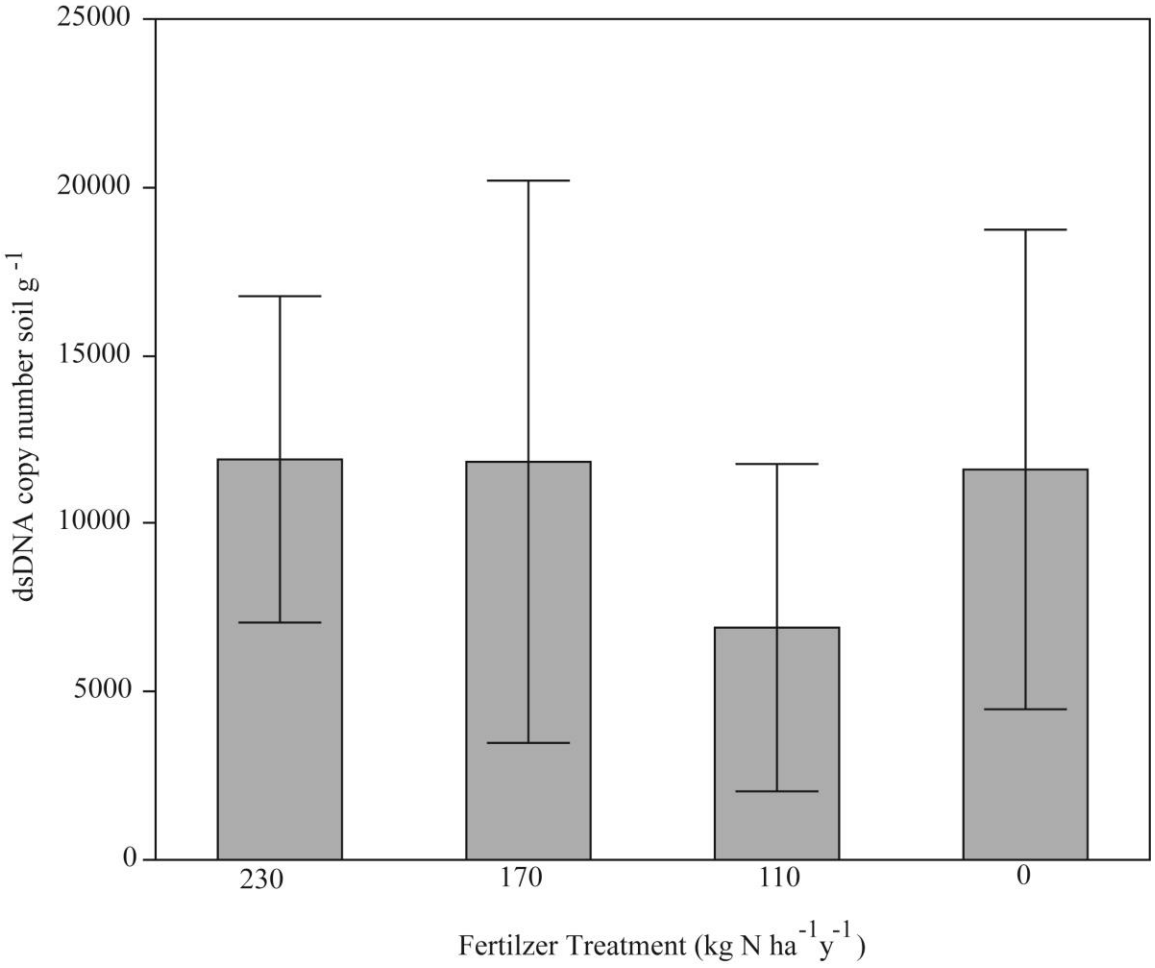

Figure 11. dsDNA copy number of AOB per soil g^{-1} under fertilizer treatment (0, 110, 170, 230 kg N ha^{-1} y^{-1}). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

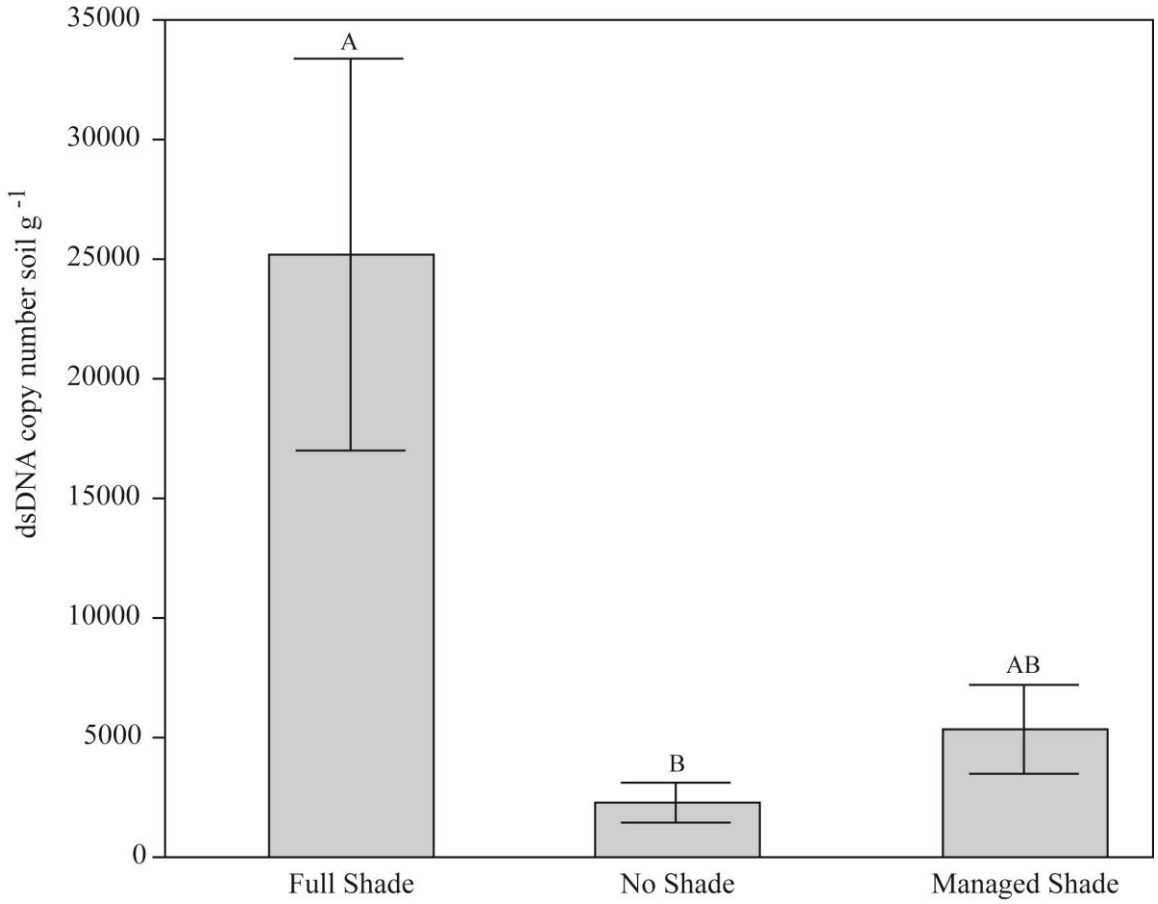

Figure 12. dsDNA copy number of AOB per soil g^{-1} by shade treatment (no shade, managed shade, and full shade). Treatments not connected by the same letter are significantly different. Each error bar is constructed using 1 standard error from the mean.

Analysis of the interaction between distances (1, 2, 3, 4, 6 and 10 m) from managed *Erythrina* trees and fertilizer treatments (0 and 230 kg N ha-1 y-1) on mean $\delta^{15}N$ was compared. No significant difference for the interaction between distance and fertilizer treatments was detected (p= 0.5389, Table 8). Thus, a full ANCOVA could be performed.

Table 8. Analysis of the interaction between distance (1, 2, 3, 4, 6 and 10 m) from managed *Erythrina* trees and fertilizer treatments (0 and 230 kg N ha^{-1} y^{-1}) on mean δ15N in mature *Coffea arabica* leaf samples.
Mean $\delta^{15}N$

Effects Test	DF	Sum of Squares	F Ratio	Prob>F
Fertilizer Treatment	1	4.9877778	10.8537	0.0024*
Distance	1	0.2383472	0.5187	0.4766
Distance *Fertilizer Treatment	1	0.1773472	0.3859	0.5389
Analysis of Variance	**DF**	**Sum of Squares**	**Mean Square**	**Prob>F**
Error	32	21.30406	1.42	0.0027*

*Indicates significance

Analysis of mean $\delta^{15}N$ was carried out by ANCOVA between distances (1, 2, 3, 4, 6 and 10 m) from managed *Erythrina* trees and fertilizer treatments (0 and 230 kg N ha^{-1} y^{-1}). No significant difference for the distance effect was determined (Prob>F 0.4724). Significant difference in mean $\delta^{15}N$ between fertilizer treatments was detected (p= 0.0022, Table 9).

Table 9. Analysis of covariance by distance (1, 2, 3, 4, 6 and 10 m) from managed Erythrina trees and fertilizer treatment (0 and 230 kg N ha^{-1} y^{-1}) on mean $\delta^{15}N$ in mature *Coffea arabica* leaf samples.
Mean $\delta^{15}N$

Effects Test	DF	Sum of Squares	F Ratio	Prob>F
Fertilizer Treatment	1	4.9877778	11.0595	0.0022*
Distance	1	0.2383472	0.5285	0.4724
Analysis of Variance	**DF**	**Sum of Squares**	**Mean Square**	**Prob>F**
Error	32	14.882764	0.45099	0.0070*

*Indicates significance

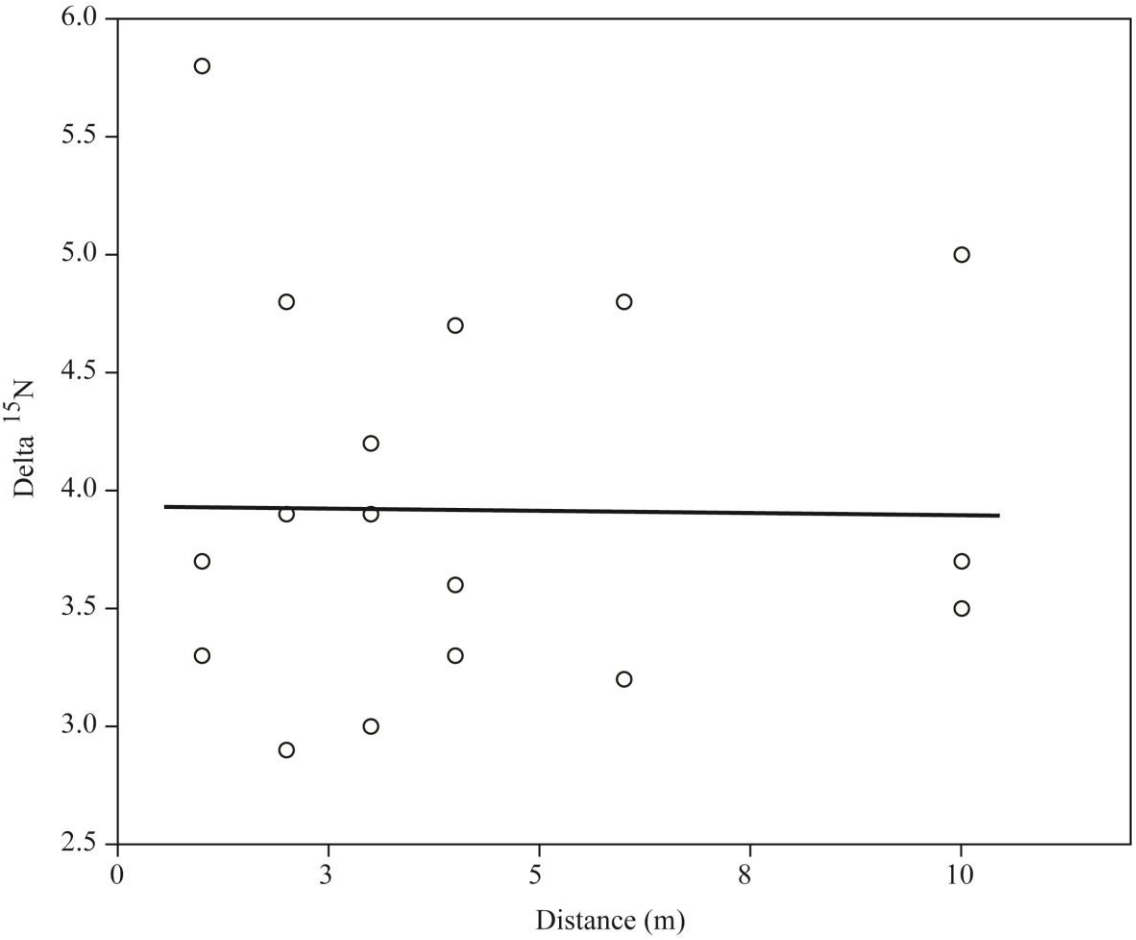

Figure 13. Regression plot of mean $\delta^{15}N$ in coffee leaves with no fertilizer treatment (0 kg N ha^{-1} y^{-1}) and distance from the managed *Erythrina* tree (1, 2, 3, 4, 6 and 10 m).

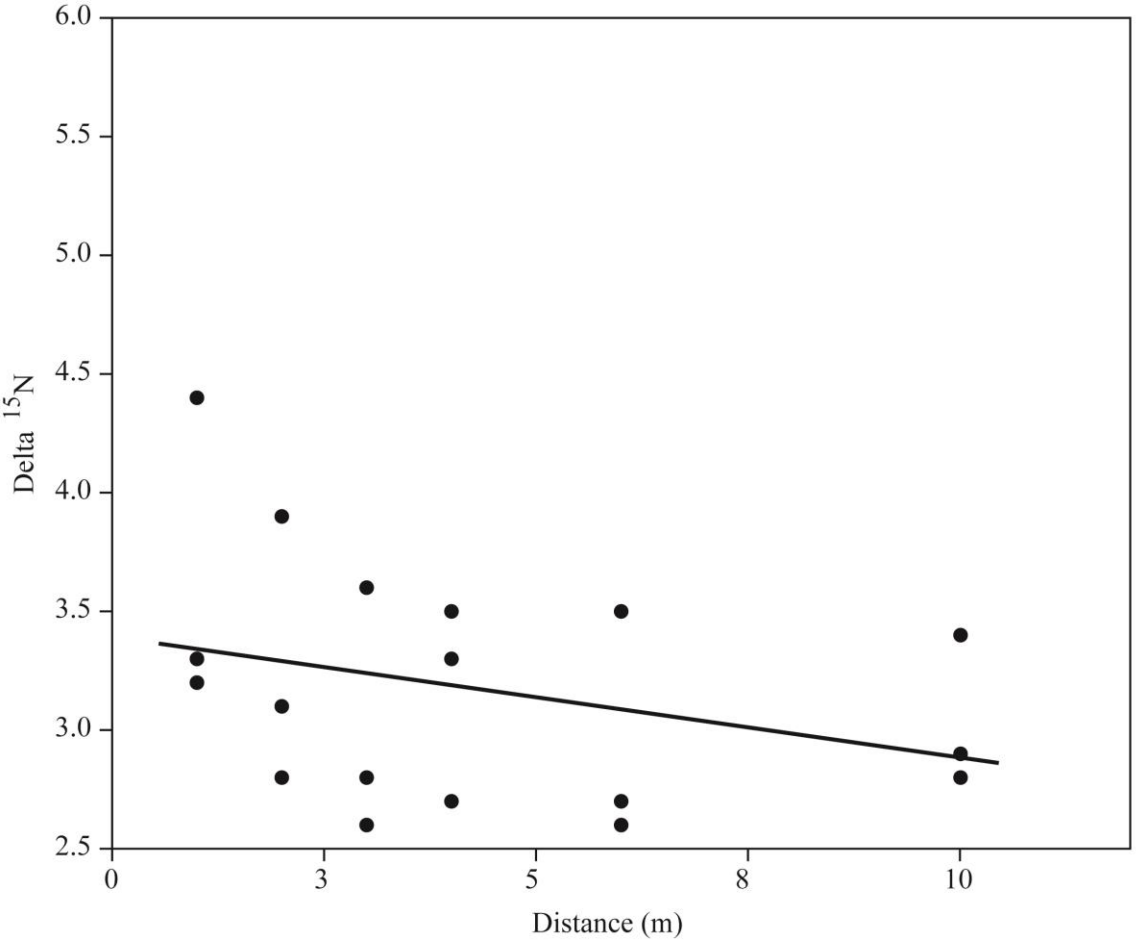

Figure 14. Regression plot of mean $\delta^{15}N$ in coffee leaves with fertilizer treatment (230 kg N ha^{-1} y^{-1}) and distance from the managed *Erythrina* tree (1, 2, 3, 4, 6 and 10 m).

CHAPTER 4

DISCUSSIONS

The primary objectives of this study were to examine how inorganic fertilizer application and the intercropping and management of *E. poeppigiana* affect soil organic carbon and nitrogen dynamics in coffee agroforestry systems in Costa Rica. While the effects of fertilizer application on crop yield and mineralization rates in coffee agricultural systems have been well studied, the effects of inorganic fertilizer application and the intercropping of *E. poeppigiana* in coffee agroforestry systems on SOC, N mineralization rates and nitrifying bacteria abundance is relatively understudied. This study evaluated fertilizer treatments (0, 110, 170 and 230 kg N ha^{-1} y^{-1}) in relation to varying rates of shade treatments (no shade, managed shade, full shade), characterized by the management style of *E. poeppigiana* planted amongst the main coffee crop.

Treatments of fertilizer coupled with shade management practice of *E. poeppigiana* did not significantly affect SOC in Mg ha^{-1} at the 0-20 cm soil depth. A higher rate of fertilizer application along with full-growth *Erythrina* trees was predicted to show a significant increase in the amount of soil organic carbon being sequestered in the 0-20 cm soil layer when compared to low fertilizer, full sun treatments. Christopher and Lal, 2007 reported higher crop production in response to mineral N fertilizer application results in greater root exudates and more crop residues, therefore enhancing SOC sequestration in agricultural soils. Although, the addition of N has also been reported to have a negative, or no effect on SOC sequestration (Mack *et al.*, 2004). In this study, the treatment of fertilizer, the treatment of shade, or the interaction of the two treatments did not significantly affect total SOC Mg ha^{-1} in the 0-20 cm soil layer. Leaf

litter accumulation on the ground was observed to be highest amongst the full shade treatment, due to the large amount of leaf deposition from the full-growth *Erythrina* trees intercropped with the coffee crop. While the addition of rich organic matter can be a significant attribute to increasing total SOC, the results of this study would suggest that the amount of organic matter being contributed by the *Erythrina* trees was not enough to cause a significant difference in total SOC (Gentile *et al.*, 2010).

The measurement of total NH_4^+ and $NO_2^- + NO_3^-$ by fertilizer and shade treatment were not significantly different by the fertilizer treatment, but did yield significantly different results by the shade treatment. The hypothesis was that the presence of the *Erythrina* trees would contribute additional N through organic material deposition and biological N fixation, ultimately increasing NH_4^+ and $NO_2^- + NO_3^-$ concentrations. In calculating total NH_4^+ and $NO_2^- + NO_3^-$, the highest concentrations were determined to be in the full shade treatment. Therefore, the observed increase in total NH_4^+ and $NO_2^- + NO_3^-$ is presumably due to an increase in leaf litter deposition, root and nodule turnover, and biological fixation by the full-growth *Erythrina* trees. Land use types have been determined to have a significant effect on NH_4^+ and $NO_2^- + NO_3^-$ concentrations, as (Neill *et al.*, 1997) determined forested land use types have higher NH_4^+ and $NO_2^- + NO_3^-$ concentrations in contrast to pasture lands. While a significant difference in NH_4^+ and $NO_2^- + NO_3^-$ concentration by shade effect was calculated, no significant difference in net N mineralization rates were observed by fertilizer or shade treatments. No significant difference in net N mineralization rates by fertilizer and shade treatments indicates the presence of full-growth *Erythrina* contributes to higher concentrations of plant available N, without increasing the risk of rapid nitrification rates and increased N pollution.

In calculating the absolute quantification of the nitrifying bacteria group AOB by fertilizer and shade treatments, a significant difference in dsDNA copy number of AOB per soil g^{-1} was determined by shade effect. The highest concentration of dsDNA copy number of AOB per soil g^{-1} was observed in the full shade treatment. The AOB nitrifying community has been observed to have a greater biodiversity in rich organic carbon environments rather than in low organic carbon environments (Racz *et al.*, 2010). Furthermore, the AOB community has been observed in high abundance in high NH_4^+ environments (Huang *et al.*, 2014). In this study, SOC was not significantly different between treatments; however, total SOC Mg ha^{-1} was high in each plot. Additionally, total NH_4^+ concentration was significantly higher in the full shade treatment. The rich organic matter and higher NH_4^+ environment of the full shade treatment may have contributed to a higher abundance of AOB when compared to the managed shade and no shade treatments.

In assessing the effects of inorganic fertilizer application on the transfer of biologically fixed N by *E. poeppigiana* to *Coffea arabica* in agroforestry systems, the hypothesis was that coffee plants closer to managed *Erythrina* would receive more N. Moreover, it was hypothesized that increased fertilizer application would negatively affect the biological N fixation in *E. poeppigiana.* The distance of coffee plants, up to 10 m, were not significantly different in mean $\delta^{15}N$. These results are difficult to interpret. If the hypothesis was correct, the results would have shown a more linear relationship, with $\delta^{15}N$ increasing with distance from the managed *Erythrina* tree. However, there is also a possibility that coffee plants in this study are receiving N from managed *Erythrina* up to a distance of 10 m in insignificantly different quantities. However, increased fertilizer

treatment had a significant effect on mean $\delta^{15}N$ between treatments, the application of fertilizer may have negatively affected N fixing capacity.

This study could have been improved by adding more sampling periods before and after chemical fertilizer treatments. It would be interesting to better document the effects of chemical fertilizer treatments with respect to time. Having a better understanding of the pathways taken and volatilization time of chemical fertilizers in coffee agroforestry systems in Costa Rica would be beneficial in reducing N pollution. Furthermore, this study may have been improved with better field preservation methods for AOB sampling. Improved DNA preservation techniques from the field sampling to the isolation process may have resulted in better qPCR detection.

CHAPTER 5

CONCLUSIONS

Ultimately, intercropping with full-growth *E. poeppigiana* is a significant, ecologically based management practice to supplement the N pool in coffee agricultural systems. By intercropping with *E. poeppigiana* in coffee production systems, inorganic fertilizer applications can be reduced, bringing down the economic cost of production for the farmer and reducing the risk of N pollution through leaching and denitrification. Future research should target quantifying the least amount of inorganic fertilizer that can be applied to an *E. poeppigiana*-coffee agroforestry system without sacrifice to crop yield. Additionally, future studies should target the absolute quantification of AOA to determine which community, AOA or AOB, are more dominant in *E. poeppigiana*-coffee agroforestry systems. A better understanding of the nitrification process and net N mineralization rates could lead to improved deliverance of plant available forms of N in agricultural systems, while reducing the risk of negative environmental effects.

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