Implications of Climate Change on the Growth of Two Tropical Agroforestry Tree Seedlings

by

Shahira Esmail

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Tropical agroforestry systems are perceived to have the capacity to be resilient to future changes in climate. This study quantifies the response of two tropical agroforestry tree seedlings; *Gliricidia sepium* (Jacq.) Walp and *Cedrela odorata* L. to increases in atmospheric concentrations of carbon dioxide (CO_2) (800 ppm), temperature (+2°C daytime and +3°C nighttime) and the combined conditions. As well, this study analyzes the microbial community structure and nutrient concentration in response to elevated concentrations of CO_2 on tropical silvopastoral soil, conventional pastoral soil and a regenerated forest soil. Both tree species demonstrated very individual responses to the different climate scenerios. While no significant CO_2 fertilizer effect was observed in either species the combined treatment demonstrated a significant increase in seedling height for both species. The response of *G. sepium* to the combined treatment was similar to its response to the temperature treatment which could be a result of achieving the optimal range in temperature for growth. As well, an increase in C:N ratio from *G. sepium* seedling leaves under the combined treatment indicates the possibility of the nutrient concentration diminishing thereby reducing the role of this species as a provider of high nutrient biomass. The soil microbial community showed very little change in response to elevated concentrations of CO_2 and differences in community structure between sites were also negligible. Soil nutrient concentration maintained the best balance over the course of both twelve week incubations for the regenerated forest site followed by the silvopastoral site and lastly the conventional pasture site. The response of soil nutrient concentration to elevated concentrations of CO_2 was negligible reflecting the response of the soil microbial community.

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Chapter 1: General Introduction

1.1 Introduction

Tropical ecosystems, characterised by their latitudinal position at 0-23.4º north and south and distinct climatic regimes, offer a multitude of services at the global and ecosystem scale. Tropical forests provide unique habitats allowing for the proliferation of biodiversity hotspots including endangered organisms and those that have not yet been identified (Chazdon, 2002; Bawa et al., 2004; Michon et al., 2007). These habitats, formed through the selection of very specific and constant conditions of temperature and precipitation, are highly sensitive to changes in climate which could impact biodiversity on a global scale (Chazdon, 2003). Due to its significant role in global processes and functions, understanding how tropical systems may react to changes in climate is vital for climate change adaptation strategies. Millions of people depend directly on the various services and products provided by tropical ecosystem to sustain their daily livelihoods. Ecological services derived from tropical systems include contributing to global hydrological cycling, global cycling of nutrients and carbon in addition to biodiversity (Bawa et al., 2004; Hutchinson et al., 2007; Michon et al., 2007).

The greatest threat to tropical ecosystems is the conversion of primary forests or grasslands to agroecosystems dominated by crops and livestock (Amezquita et al., 2005). This change in land use has resulted in a decline in biodiversity, soil degradation, habitat fragmentation and has negatively affected carbon (C) and nutrient cycles (Esquivel et al., 2008). The implementation of sustainable agricultural land management practices, such as agroforestry systems, may help address the current and prevalent issues relating to land degradation and soil infertility (Esquivel et al., 2008). Agroforestry is defined as the inclusion of woody perennial species within an agricultural land area, including crop and/or

pasture dominated use (Nair, 1993). Due to their complex structures agroforestry systems help to maintain diversity on the landscape, levels of soil organic matter and soil fertility. The higher input of organic matter from tree pruning, leaf litter and root turnover within an agroforestry system compared to monocrop agroecosystems maintain levels of soil organic matter (SOM) on the landscape. Soil organic matter contributes to soil fertility and has the potential to sequester C over the long-term (Oelbermann et al., 2004; Esquivel et al., 2008).

Global ecosystems are currently subjected to a new disturbance: namely climate change. Irrespective of cause, it is indisputable that current research demonstrates an overall warming of the global climatic system including higher average air temperatures, ocean temperatures, increased rates of rising sea levels and increased rates of ice melts (IPCC, 2007). Elevated levels of greenhouse gases (GHG) in the atmosphere has lead to warming trends and events which begin positive feedback loops within the system exacerbating current conditions (IPCC, 2007). Changes in climate do not only signify a change in average temperature but will alter precipitation, occurrence of extreme weather events, wind patterns and ultimately all aspects of the natural resources upon which all global life depend (IPCC, 2007). This includes the probability of impacting levels of agricultural productivity under current management practices. Climate change models suggest different scenarios and predictions which are predominantly presented within a global context, however; the true impacts will occur within a local context. Field and laboratory research can be used as a tool to further understand the ramifications of the changing climate on localised scales and for economically relevant land uses such as agriculture, thereby reducing the vulnerability of differing communities to these impacts with the aim to increase their capacity to adapt to climate change.

1.2 General Research Goals and Objectives

There is uncertainty as to whether agroforestry systems will remain effective at providing the products and services necessary for sustainable agriculture when exposed to a changing climate. In order to evaluate the impact of climate change on these systems, the response of the individual components to these conditions must be understood.

The purpose of this study is to determine how tropical agroforestry tree seedling growth will be effected by the expected change in temperature and atmospheric concentrations of carbon dioxide (CO_2) associated with climate change in order to provide further insight to help inform adaptation strategies for agroforestry systems.

The general objectives of the study are as follows:

1) Quantify changes in tree seedling growth rates under ambient conditions, an elevated CO_2 concentration treatment, an elevated CO_2 concentration in combination with the resulting change in temperature, and the temperature change only.

2) Quantify changes in the carbon and nitrogen concentrations of the soil under an elevated concentration of CO_2 compared to an ambient treatment.

3) Determine changes in soil microbial community structure under an elevated concentration of CO_2 compared to an ambient treatment.

Chapter 2: Literature Review

2.1 Potential Implications of Climate Change on Tropical Agroecosystems

Global modelling studies have demonstrated that concentrations of atmospheric carbon dioxide (CO_2) have been rising at an unprecedented rate over the past century and predict rates could more than double over the next century to over 750 ppm (Cox et al., 2000; White et al., 2000; Ometto et al., 2005). Temperature changes as a result of increased concentrations of greenhouse gases (GHG) including CO_2 has resulted in a 0.6º C increase in mean global air temperature over the past century (IPCC, 2007). Predictions for the future include night time temperatures increasing more rapidly than daytime temperatures (IPCC, 2007). For example, in tropical regions temperature has increased 0.26ºC per decade from 1976-1998 (Malhi & Wright, 2004). It is predicted that temperatures could increase by 2.5 to 2.9ºC within the tropics over the next century corresponding to similar increases in CO_2 (White et al., 2000; Zhang et al., 2001). Current predictions include a short-term rapid increase in temperature that does not follow the previously predicted linear increase (White et al., 2000). As well, positive feedback loops must be taken into account. For example, increased temperatures in tropical forests causes elevated rates of biological respiration thereby resulting in increased levels of CO_2 released and increasing atmospheric GHG (Graham, 2003). However, these feedbacks are complex and not fully understood (Graham, 2003). These changes in climate could impact the capacity for vegetation to grow within the tropics, including the trees associated with agroforestry systems (Clark, 2004).

Specifically, ecosystem functions influenced by climate are expected to be affected. This includes species distribution and extinction rates, biome shifts and biogeochemical cycling (Williams, 2007). Within an agricultural setting the direct impacts could include a change in the optimal range for vegetation growth and resiliency to disturbance such as pests

(Williams, 2007). Changes in levels of precipitation and occurrences of drought and floods could impact the availability of feed for livestock, levels of crop productivity and may alter decomposition rates leading to a change in soil fertility and nutrient availability (Sanchez, 2001; Williams, 2007). As well, any change in climate could alter the timing for flowering, fruiting, seed production, and germination of crops (Bazzaz, 1998). While this may be location and species specific, the potential ramifications could be significant for people directly dependent on agriculture. Sustainable agricultural practices, such as agroforestry, have the potential to provide one means of adapting to a changing climate.

2.2 Tropical Agroforestry Systems

2.2.1 Historical Perspective

Traditional agricultural systems have historically utilized a wide variety of management practices including the incorporation of multiple crops (intercropping) or integrating trees with crops and/or pasture (agroforestry) on the same land unit at the same time (Nair, 1993). The purpose for such complex agroecosystems is varied, and includes the need for timber and fuel wood, production of food and livestock fodder, products derived from trees and the beneficial interactions between crops, trees and soil (Scales and Marsden, 2008). Some of the earliest examples include slash and burn techniques and home gardens dating back to more than 7000 years ago (Scales and Marsden, 2008; Lentz and Hockaday, 2009). As well, there is evidence of agroforestry being used by the Mayan's over 1000 years ago, using mainly *Manilkara zapota* (L.) P. Royen trees, commonly known as sapodilla, which has hard, durable and very wear resistant wood for the construction of palaces and ceremonial buildings. These trees are believed to have been maintained in grooves with important vegetation such as fruit bearing trees and spiritually significant species (Scales and Marsden, 2008; Lentz and Hockaday, 2009). Varying agroforestry techniques were used up

to the beginning of the last century in Europe and other temperate regions, however monocultures quickly gained prevalence in that region thereafter (Torquebiau, 2000). Agroforestry continued to be utilized especially in tropical regions during that time with examples such as the use of *Erythrina poepiggiana* (Walp.) OF Cook trees for shade grown coffee (*Coffea Arabica* L.) and cocoa (*Theobroma cacao* L.), the rubber tree (*Hevea brasiliensis* Müll.Arg.) agroforests of Indonesia and the coffee forests in Ethiopia (Torquebiau, 2000).

Agroforestry became a recognized science in the early 1970's as a result of greater awareness and concern over global environmental issues (Pollini, 2009). Following the Green Revolution, there was a focus on increasing agricultural production levels for subsistence farmers, however there was also a new understanding that one method could not be employed successfully in all regions (Pollini, 2009). Research conducted by the International Development Research Centre (IDRC) of Canada recommended the creation of the International Council for Research in Agroforestry (ICRAF), now known as the World Agroforestry Centre, in 1977. It is through this centre that extensive consistent research in agroforestry practices were conducted and extension for sharing new techniques became common (Pollini, 2009; ICRAF, 2009).

2.2.2 Defining Agroforestry Systems

Agroforestry systems, according to the World Agroforestry Centre, are defined as the deliberate spatial and temporal incorporation of woody perennials with agricultural land use or practice including crops and/or livestock. The interactions between woody and non woody components on the land can have both ecological and economic benefits and that there is a variety of forms that this incorporation can take (ICRAF, 2009). A more thorough definition available is;

"a dynamic, ecologically based, natural resources management system that, through the integration of trees on farms and in the agricultural landscape, diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels." (ICRAF, 2009)

Various types of agroforestry systems exist in tropical biomes and include the incorporation of trees at different densities, configurations, and the use of different tree species. Techniques can be divided into three dominant groups; agrosilvicultural systems which incorporate crops and trees together, agrosilvopastoral systems where trees, crops and livestock are incorporated in a pasture or non pasture setting and silvopastoral systems where trees and livestock are incorporated together (Nair, 1993; Torquebiau, 2000).

Agrosilvocultural systems include practices such as alley cropping in which alternating rows of food crops are planted with shrubs or hedgerows of woody perennial species that are often nitrogen (N) fixing (Nair, 1993). The presence of N fixing species adjacent to crops, the input of biomass from pruning and the possibility of incorporating value added products such as fruit or nuts has been demonstrated to improve soil fertility and productivity (Nair, 1993; Molua, 2003). Alley cropping has also been found to maintain or improve grain yield and sequester greater amounts of carbon (C) in the soil when compared to conventional monoculture systems in tropical environments (Oelbermann, 2004). Another example includes improved fallow where fast growing short rotation trees are utilized in rotation with crops or pasture land to improve soil fertility and N levels (Wilkinson, 2007).

Silvopastoral systems refer to the incorporation of trees in a pastoral land management system. The role of trees in these systems is diverse and includes providing shade for livestock, provision of alternate sources of forage and fodder, live fences, mulch to maintain soil fertility and grass productivity and sources of alternate income through timber,

fruit and nut production (Bambo et al., 2009; Pollini, 2009). Silvopasture has also been shown to be beneficial for livestock through the occurrence of reduced parasites and the promotion of increased feeding and activity (Francisco et al., 2009; Karki and Goodman, 2010). Some of the benefits of integration of trees into pastureland include increases in above and belowground plant productivity (Scholes and Hall, 1996; Archer et al., 2001), modifications to rooting depth and distribution (Gill and Burke, 1999), and changes in the quantity and quality of litter inputs (Connin et al., 1997; Jackson et al., 2000; Jobba´gy and Jackson, 2000).

Agrosilvopastoral systems include the incorporation of crops, pasture and trees on the same land use. This often represents a complex system where trees are utilized as a protective barrier either for wind or in riparian zones, thereby protecting waterways (Nair, 1993). It has been argued that this category can technically include any form of agroforestry, including those mentioned above due to the temporal aspect (Torquebiau, 2000). However, one example of all three components being incorporated on a landscape at the same time is home gardens. Typically, home gardens are perennial and annual species planted for the purpose of livelihood supplementation and can include livestock. Home gardens have the potential to assist in biodiversity conservation through the presence of a greater diversity of native plant species and endangered or rare species when compared to other agricultural settings (Webb and Kabir, 2009).

2.2.3 Biogeochemical cycle and Benefits of Agroforestry Systems

The implementation of woody perennial species on agricultural land impacts all of the components of the system including the soil, crops and the interactions with other systems including the atmosphere (Figure 2.1) (Torquebiau, 2000). The soil component can be divided into physical, chemical and biological characteristics. All categories contribute

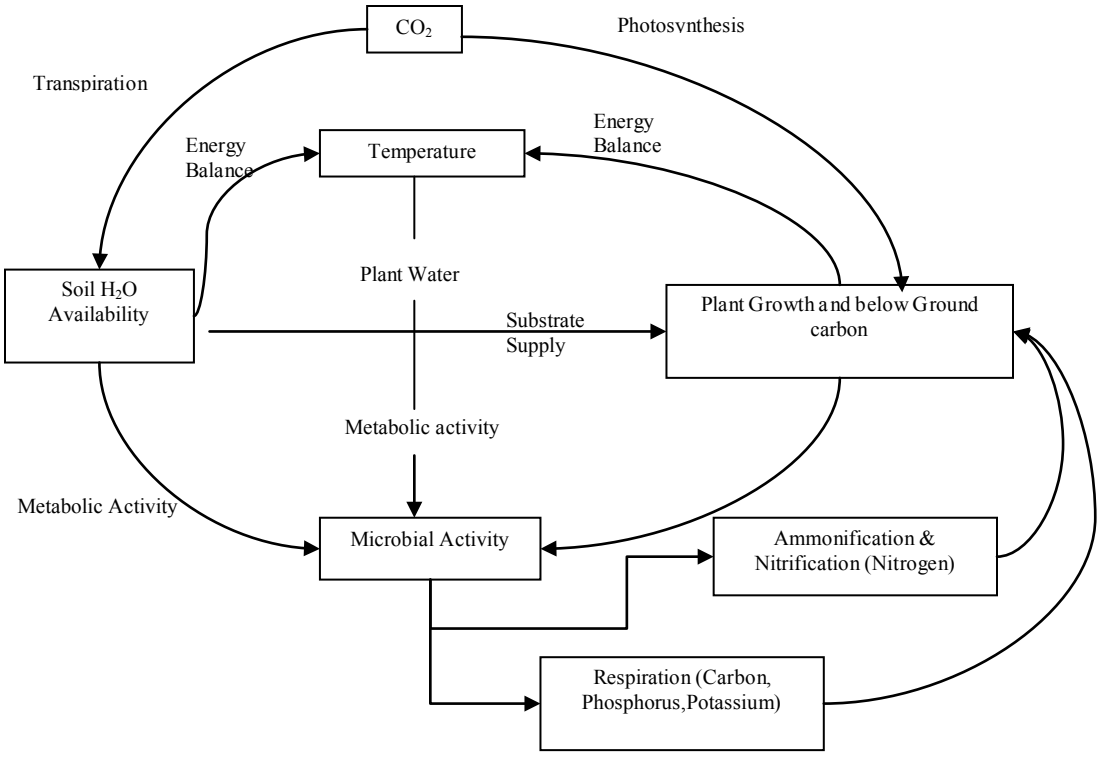

Figure 2.1 Example of a biogeochemical cycle for an agricultural system incorporating the soil, plants and the atmosphere (Adapted from Wan et al. 2007)

to the fertility or possible productivity of the land. One of the main indicators of fertility is the presence of soil organic matter (SOM) available to provide valuable nutrient for the growth of crops and trees (Manlay et al., 2007). Soil organic matter can be divided into three pools. The active pool represents the microbial biomass and materials with a high turnover rate which could be representative of yearly fluctuations due to crop harvest (approximately 1.5 yrs). A slow pool includes materials more resistant to physical and chemical break up with a turnover of about 25 years. Finally, a passive pool contains recalcitrant or very resistant compounds which could take 1000 years to decay (Parton et al., 1987; Manlay et al., 2007). Decomposition is one of the main processes contributing to the levels of SOM. Decomposition within soil is a function of microbial activity where non living tissue and

constituents are physically and chemically transformed ultimately releasing energy and nutrients into bioavailable forms to facilitate crop growth (Milton and Kaspari, 2007). Tropical soils are especially sensitive to the rapid depletion of SOM (Maia et al., 2006). The implementation of intensive conventional agricultural practices, such as high rotation monocroping systems, initially results in high production levels. However, within a few years the active SOM levels are reduced due to the high rates of decomposition and with it the many nutrients required to maintain production levels (Maia et al., 2006). Erosion, a physical process resulting in the loss of nutrient rich top soil predominantly through wind and water is another cause for the loss of productivity in many tropical intensive agricultural management systems (Nair, 2009).

Through the addition of trees on agricultural land, agroforestry has the potential to mitigate some of the causes of the degradation of land in conventional agricultural management systems (Jackson et al., 2000; Nair, 2009). Biomass inputs of tree roots, leaves and branches can replenish SOM levels and when combined with techniques such as improved fallow are able to maintain long term fertility (Jackson et al., 2000; Jobba´gy and Jackson, 2000; Jose, 2009; Nair, 2009). Often agroforestry utilizes N_2-fixing tree species which help maintain soil N levels and deep rooted trees are able to utilize nutrients available at greater depths making these nutrients biologically available for crops or forage grasses (Nair, 1993; Rao et al., 1998; Gill and Burke, 1999; Ong et al., 2001). For example a study by Bambo et al. (2009) demonstrated that silvopastoral systems in Florida maintained a great retention of N in soils when compared to conventional pastures through a significant reduction in N leaching. In another study, by Lee and Jose (2005) where a pecan (*Carya illinoinensis* Wangenh. K.Koch) and cottonwood (*Populus deltoides* W Bartram ex Marshall) alleycropping was examined, higher levels of soil microbial biomass and SOM

were found compared to a monoculture of cotton. Agroforestry also has the potential to improve physical soil characteristics. In a study by Kumar et al. (2010) in Missouri it was shown that soil porosity levels in soil 0-10 cm deep could increase up to 13 times more under buffer strips of cottonwood trees (*Populus deltoides* W. Bartram ex Marshall ssp. *deltoides*) as compared to red clover (*Trifolium pratense* L.) and Korean lespedeza (*Kummerowia stipulacea* (Maxim.) pasture.

Another aspect to agroforestry systems are the interactions which occur between trees and crops or pasture grasses. Most plants have similar basic requirements at varying quantities for growth and survival such as nutrients and moisture through the soil component, atmospheric components such as oxygen, CO_2 and sunlight (Pollini, 2009). When the study of agroforestry first began there was great focus on the potential detrimental competition between tree and crop roots (Pollini, 2009). However, various studies have demonstrated that in most cases trees can access a greater depth of soil than crop roots can attain thereby improving nutrient availability and soil structure (Nair, 2009). Also, many studies have found environmentally beneficial interactions of tree-crop roots in the upper soil levels (0-20 cm) through the reduction of erosion rates and leaching of excess nutrients (Allen et al., 2004). While some competition does occur for soil moisture and excess shade could have negative effects in growth, changes in management techniques and species selection is able to maximize facilitation and minimize competition (Gea-Izquierdo et al., 2009; Pez- Diaz et al., 2009).

The diversity and structure which agroforestry provides also influences water quality, biodiversity and C sequestration (Allen et al., 2004; Jose, 2009). The ability of tree roots to access excess nutrients at greater soil depths serves as a safety net to reduce leaching and surface runoff of sediment and applied chemicals thereby reducing contamination of local water sources (Allen et al., 2004; Bambo et al., 2009). The presence of trees on the

landscape is able to act as a mechanism of biodiversity preservation. Certain spatial configurations of trees can act as corridors for species movements between patches of habitat or can act as habitat for species less sensitive to disturbances. As well, agroforestry is increasingly recognized for its ability to preserve germplasm of sensitive species (Jose, 2009). The diversity and complexity of agroforestry systems provides the potential for microhabitats thereby increasing the diversity in soil fauna such as in shade cacao systems (Jose, 2009). A study by Jha and Vandermeer (2009) demonstrated that shade coffee agroforestry systems in Mexico had the potential to provide greater habitat for native social bees and native wasps compared to invasive species. While there are numerous other interacting components in agroforestry systems, the final discussion will pertain to the potential of agroforestry for C sequestration. Carbon sequestration is the removal and storage of atmospheric CO_2 into sinks through biological and chemical processes (Jose, 2009). Carbon is captured during tree growth and stored in plant biomass which can contribute to overall levels of atmospheric CO_2, however recent focus has shifted to root and soil storage of C (Nair et al., 2009). Soil reserves of C are three times the levels in the atmosphere therefore it has the potential to be a significant source of CO_2 or an even greater sink. Soil C sequestration is facilitated by plant photosynthesis and the conversion into soil organic C (SOC). The higher the level of biomass input reflects a higher potential in SOC sequestration. For this reason agroforests are ranked as the second highest land use for levels of SOC after old growth forests (Nair et al., 2009). However in all instances variability in benefits exist due to countless factors such as climate, geology and the specific management techniques chosen.

2.3 Effects of Climate Change on Tropical Agroforestry Systems

2.3.1 Influence on the Growth of Trees

Liebig's Law of Minimum states that the growth of all plant life is determined by the most limiting component; be it water or nutrients or any other factor (Liebig, 1831). In the past it was hypothesized that CO_2 may be a limiting factor in photosynthesis and that an increase in CO_2 levels would further drive accelerated rates of photosynthesis; thereby increasing growth and biomass accumulation, also known as the CO_2 fertilization effect. This has been found to be true, especially for C_3 plants including trees which respond up to a level of 800 ppm of atmospheric CO_2 before levelling off. C_4 plants, including corn, had a much lower response with the maximum rate of photosynthesis occurring at 400 ppm of CO_2 (Akita & Moss, 1973; Notoro et al., 2007). The CO_2 fertilization effect is a function of stomatal conductance, where higher concentrations of CO_2 allows for a more narrow opening of the stomata thereby conserving water and decreasing respiration resulting in greater water use efficiency (Korner, 2000; Notoro et al., 2007). In more temperate regions elevated concentrations of CO_2 enhanced biomass production to varying degrees in seedling growth depending on species type. For example fast growing, early successional trees such as aspen (*Populus tremuloides* Michx.) experienced a greater increase in biomass including root growth and leaf size than slower growing trees (Lindroth et al., 1993). However, it has also been shown that the impacts of CO_2 fertilization are the most pronounced in drought condition where the optimization of water use efficiency causes the greatest increases in photosynthesis, while in water rich conditions the effects may be inconsequential (Hamann & Wang, 2006).

Early studies demonstrated a drastic decrease in respiration in response to elevated CO_2 due to its inhibitory effect on the enzyme *cytochrome c oxidase* necessary within the

electron transport chain; however more recent investigation has shown this decrease is due to experimental error (Chambers & Silver, 2004). The investigation of the inhibitory effects of CO_2 on *cytochrome c oxidase* continues and at present it is believed that a decrease in respiration of 10% may occur (Hamilton et al., 2001).

Studies within the tropics have demonstrated varying degrees of the CO_2 fertilization effect depending on limiting nutrients (Lovelock et al., 1999; Chambers and Silver, 2004). However, the possibility of excess CO_2 absorption causing detrimental effects or no significant increase in biomass has been observed (Lovelock et al., 1999; Chambers and Silver, 2004). Other field experiments with tropical rainforest tree seedlings exposed to elevated CO_2 have shown variable response depending on the species involved and when there was an increase in growth rate it diminished over the long term (Clark, 2004).

Temperature also has the potential to influence the growth of trees within the tropics. One study by Cavaleri et al. (2008) examining old growth tropical rainforests found that as temperature increased the rates of respiration from the leaves increased as well. During El Nino Southern Oscillation years with higher temperatures respiration was found to be about 9% greater than other years. As well, it was acknowledged that measurements during La Nina caused uncharacteristically low values for respiration thereby impacting the estimates of these trees as C sinks (Cavaleri et al., 2008). Temperature can also impact rates of photosynthesis; having a positive effect up to a threshold and then causing very rapid declines in growth (Keller & Lerdau, 1999; Clark, 2004). The effect of temperature alone by raising respiration rates and decreasing photosynthesis could lead to an overall decrease in net primary production (NPP) and a decline in potential to store C (Nemani et al., 2003).

The combined impact of the rise in temperature and CO_2 has some variable predicted responses. CO_2 fertilization effect is believed to vary with differing temperatures; as temperature increases the oxygenation reaction catalyzed by *Rubisco* has a competitive

advantage over the carboxylation reaction catalyzed by the same enzyme; as well oxygen is more soluble than CO_2; therefore the increase in photosynthesis outweighs the increase in respiration (Chambers & Silver, 2004). One approach to determine the interactive effects of the changing climate on the growth of trees have been to monitor long term plots over time to determine any changes realized to date on tropical forest species. A study by Lewis et al. (2004) examined fifty different forest plots located throughout South America from 1971-2002 to determine how the tropical forest systems were changing over time. Over the thirty years Lewis et al. (2004) showed that there was an increase in stand level basal area, basal area growth rates and mortality, as well as a similar trend in woody stems. However, the overall increase in growth outweighed the losses in biomass reflecting an overall increase in biomass throughout the various study plots. The overall increase in biomass is a reflection of the availability of resources and the interaction of various components; however it does not necessarily reflect changes that may occur in the coming years.

2.3.2 Influence on Soil Microbial Communities and Nutrient Dynamics

The growth of agroforestry trees is deeply reliant on and directly impacts the soil components including the availability of soil nutrients, soil organic carbon and the microbial communities within (Pollini, 2009). Not only will the trees be impacted by the change in climate but both the soil microbial communities and therefore the resulting dynamics of nutrients will also be affected (Pollini, 2009).

A direct correlation between the effects of temperature on soil microbial activity has been comprehensively studied and is well understood; demonstrating an increase in enzymatic activity and chemical reactions facilitating this activity up to a threshold temperature (Wu et al., 2010). Soil respiration is a function of microbial activity which is influenced by temperature; however, rate of soil respiration is also dependent on other

variables including availability of substrates, moisture and quality of substrate. Vegetation type has minimal impact on the rate of respiration; however it directly contributes to the substrate quality which is highly influential (Rustad & Fernandez, 1998; Raich & Tufekcioglu, 2000). A meta-analysis of 32 different investigations revealed an overall increase in N mineralization (46%) and soil respiration rates (20%) when exposed to elevated temperatures in varying ecosystem types including forest, tundra and grassland (Rustad et al., 2001). A long term study by van Meeteran et al. (2008) demonstrated an initial increase in litter mass decomposition; however, over the long term decomposition was actually reduced due to reduced soil moisture resulting from elevated temperatures. These conditions, over the long term, also reduced N and phosphorus (P) immobilization and therefore biological availability (van Meeteran et al., 2008).

The evaluation of the impact of elevated concentrations of CO_2 on soil processes has been more difficult to determine because the soil C pool is more difficult to monitor in a field setting (Pendall & King, 2007). An open-top chamber experiment in the Colorado shortgrass steppe using stable isotopes ($\delta^{13}C$) by Pendall and King (2007) demonstrated that at elevated concentration of CO_2 surface C in both the active and slow pools initially expressed an increase in decomposition rates; however over the long term levels became stable. With the aid of the stable isotopes it was distinguished that elevated concentrations of CO_2 did not alter decomposition rates of newly inputted biomass (Pendall & King, 2007). Another incubation study, however, demonstrated very definitive changes in decomposition rate of spring wheat (*Triticum aestivum* L. cv. Triso) residue (Marhan et al., 2008). The treatments without visible residue resulted in similar mineralization rates between the ambient and elevated CO_2 conditions but the treatments with visible spring wheat residue had an

approximately 20% decrease in mineralization rates for the elevated CO_2 versus the ambient conditions (Marhan et al., 2008).

An understanding of how soil constituents including microbial communities and activity will respond to the changing climate is an area of research which requires further development (Marhan et al., 2008). There is very little understanding on the interactive effects of the individual variables associated with climate change such as temperature, CO_2, moisture and other contributing variables (Marhan et al., 2008). Research to date suggests that temperature and CO_2 enrichment cause opposing results. For example, an increase in temperature could cause an increase in microbial activity such as respiration up to a threshold level (Marhan et al., 2008; van Meeteran et al., 2008; Wu et al., 2010). However, elevated concentrations of CO_2 may cause a decline in decomposition and mineralization (Marhan et al., 2008; van Meeteran et al., 2008; Wu et al., 2010). These implications appear to be constant, irrespective of the vegetation type. Without direct research based evidence it is difficult to determine which variable may be more influential or if the response to the interaction is an unconsidered alternative.

2.4 Areas for Knowledge Improvement

Research to date involving the implications of climate change on agroforestry trees is extremely limited. In addition to this gap in research the information currently available on the interactive effects of CO_2 and temperature on the growth of trees and soil dynamics appears somewhat conflicting and uncertain. While the physiological responses of some plants are well understood under individual treatments (Clark, 2004; Hamilton et al., 2001; Keller & Lerdau, 1999; Lindroth et al., 1993), the combined effect requires greater attention on both the individual species basis, due to the variability in response, and on an ecosystem or stand level (Clark 2004; Lewis et al., 2004). The effect of temperature on soil processes is

well understood but further investigation on the effect of CO_2 is required. Although the importance of the tropical rainforest as a C sink is of great concern, systems directly tied to the livelihoods of people all over the tropics such as agriculture and agroforestry require a more close inspection.

2.5 Specific Objectives of the Study

1) To quantify the effect of elevated CO_2, temperature and a combined treatment (temperature and CO_2) on the growth of *Cedrela odorata* and *Gliricidia sepium*, multipurpose agroforestry tree seedlings.

2) To determine the effect of elevated CO_2 on the soil microbial community dynamics from three different land uses (conventional pasture system, silvopastoral system and a regenerated forest).

3) To determine the effect of elevated CO_2 on soil C and N concentration of three different land uses (conventional pasture system, silvopastoral system and a regenerated forest).

2.6 Hypotheses and Null Hypotheses

1) The CO_2 treatment will cause a significant increase in tree seedling growth due to the fertilization effect when compared to the other treatments of ambient conditions and elevated temperature.

   Ho: There will be no significant difference in growth of the two agroforestry tree seedlings between treatments.

2) The pasture site will express a significant difference in soil microbial community structure between the ambient and CO_2 treatment.

   Ho: No significant differences will be seen for soil microbial community structure between the ambient and CO_2 treatment for any of the sites.

3) The silvopastoral system and regenerated forest will demonstrated the greatest capacity to maintain nutrient levels under elevated CO_2 conditions.

   Ho: There will be no significant difference in nutrient quality between the pasture, silvopasture and regenerated forest sites.

Chapter 3: Study Site

This research is representative of agroforestry in a tropical setting; however the focus site is Esparza, Costa Rica. Costa Rica was chosen due to its wide use of silvopastoral systems and the close relationships between farmers and educational institutions encouraging sustainable agricultural practices in the farming community. Due to previous world scrutiny of the country's environmental standards, Costa Rica has become one of the world leaders in environmental legislation and sustainable agricultural practices (Quesada, 2009).

Costa Rica is located in Central America between Nicaragua and Panama. In 2008 agriculture represented just less than 10% of the country's GDP, employed over 10% of the population, and the food industry accounted for over 30% of the total exports (Ministerio de Agricultura y Ganaderia, 2008). Prominent products are melons (*Cucumis melo* L.), bananas (*Musa acuminate* L.), sugar cane (*Saccharum officinarum* L), pineapple (*Ananas comosus* L. Merr) and milk (Ministerio de Agricultura y Ganaderia, 2008). The economic importance of the agricultural industry provides the reason for investigating any possible causes for changes in productivity.

3.1 Biophysical Description of Visited Sites

Esparza, Costa Rica is located at 09°59‟ N and 84°38‟ W in the Barranca district situated in the northern area of the Puntarenas province. This region is approximately 432 km^2 and includes the cities and towns of: Esparza, Artieda, Angostura, Salitral, Salinas, Miramar, Marañonal, San Jerónimo, San Juan, Sabana Bonita, Mesetas, Macacona, San Miguel, Cerrillos, Guadalupe and Peñas Blancase. Elevation changes in this region range from 50 to 1000 m above sea level. The agricultural activity in this area is predominantly

beef production (80%), followed by mixed livestock farms (18%) and milk production (2%) (Orozco, 2002).

Figure 3.1: Study site Esparza, Costa Rica the location of the farms included in field sampling (Google Maps, 2010)

Esparza, Costa Rica is located in the sub-humid tropical forest zone (Jesus, 2008). This zone is characterized by two seasons, the wet season occurring from May to November and the dry season from December to April. As well, this region is influenced by large scale inter-annual fluctuations such as El Nino-La Nina events which impact all of Latin America. The mean annual temperature is 27.2°C with mean annual precipitation between 1500 to 2000 mm. The mean daytime temperature is 32° C and the mean nighttime temperature is 22° C. This area experiences a relative humidity ranging from 65% to 80% (Casasola et al., 2004; Auquila, 2005).

The geology consists of alluvial sedimentation and volcanic origins. The alluvial sedimentation is divided into the Esparza and Orotina terraces, and the remainder of the flat surfaces originated by alluvial fan mud currents of the Barranca, alluvial plain of the river Jesus María and marshes (Jesus, 2008). The unit of volcanic origin is divided in two denominated subunits of ridges in the mining mountain range of Tilarán and hills and valleys of the Aguacate (Jesus, 2008).

The sites for soil sampling include a conventional pasture site, silvopastoral site and regenerated forest. The conventional pasture site supports cattle for dairy and meat production. Pasture rotation between the wet and dry season is the only management technique used and common pasture grasses are used (*Panicum maximum* Jacq., *Hyparrhenia rufa* (Nees) Stapf, *Paspalum notatum* Flugge *etc.*). This site has been used with the current practices for 20 years. For this site the bulk density is 1.10 g/cm^3 and the soil texture is loam (Ramirez, 2006). The silvopastoral site is used primarily for the raising of dairy heifers. At this site live fences and fodder banks have been incorporated into the pasture setting, as well as the use of nitrogen fixing grasses. The fodder banks began 15 years ago and the live fences have been in growth soon after that. The bulk density at this site is 1.04 g/cm^3 and the soil texture is clay loam (Ramirez, 2006). The regenerated forest is adjacent to the

silvopastoral site. It was previously a conventional pasture site and has been regenerating for 14 years. Almost full canopy and vertical stratifications have been established. The bulk density at this site is 1.11 g/cm^3 and the soil texture is loam (Ramirez, 2006). The soils in all three sites were dominated by Nitosols (FAO, 1974; Ramirez, 2006). Nitosols are characterized by dark red, brown or yellow color and the presence of an argillic B horizon. This soil, within 125 cm from the surface, lacks ferric and vertic properties (FAO, 1974).

3.2 Historical Context

In 1997 Costa Rica implemented national level payments for environmental services program (PES) or *pagos por servicios ambientales*. Payments for environmental services provides a means of designating a monetary value to environmental services and a direct payment to the land user, thereby creating a financial incentive for providing these services (Pagiola et al., 2004; Pagiola et al., 2005; Sanchez-Azofeifa et al., 2007; Jesus, 2008). The first phase was from 1997-2001 and focused on forest conservation practices in an attempt to recover some of the forest area destroyed for lumber extraction. The services which were compensated were greenhouse gas mitigation, hydrological services, scenic value and biodiversity (Sanchez-Azofeifa et al., 2007). During this phase the services were not valued separately but assumed to occur together and it was based on a first come first serve process irrespective of farm size or any other characteristic of the farm (Pagiola et al., 2005). The average return was between US $22 and US $42/ha/year (Sanchez-Azofeifa et al., 2007). The second phase was initiated in 2001 and is still continuing, attempts to provide a more site specific program allowing for the inclusion of more services and altering the payments based on the specific conditions (Sanchez-Azofeifa et al., 2007).

The region of Esparza was one of the three chosen locations involved in a specific pilot PES project in Latin America along with Quindío, Columbia and Matiguás-Río Blanco,

Nicaragua. This project was initiated in 2002 as the Regional Integrated Silvopastoral Ecosystem Management Project (RISEMP) and was funded by the Global Environmental Facility (GEF) (Pagiola et al., 2005). The goal of this project is to improve degraded pastureland through the implementation of features on the landscape which would encourage biodiversity, carbon sequestration or the use of more sustainable management techniques. Some examples include the use of trees as live fences, trees as shade areas, riparian zones to protect stream water from animal waste runoff, and the use of nitrogen fixing legumes and grasses in the pasture. At the Esparza location researchers from the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) evaluated initial conditions of each farm and worked with farmers to develop specific implementation strategies. Progress assessment and evaluation was also carried out by CATIE (Pagiola et al., 2004).

The involvement of the region of Esparza in the PES project ensured accurate and comprehensive data on the initial and continuous conditions of the participating farms. The availability of recorded data made this region an ideal location for this research. Continued relationships between CATIE and the farmers provided for access to the sites and important site information from the farmers themselves. As well, the variety and extensive use of silvopastoral systems in this location made this research applicable and valuable for them.

Chapter 4: Implications of climate change variables on the growth of tropical agroforestry seedlings

4.1 Introduction

Trees have an important role in tropical agricultural systems, characterizing one form of sustainable management technique, agroforestry. While a diversity of roles exist for trees on an agricultural setting, silvopastoral systems have proven to be easily implemented and economically and ecologically beneficial (Nair, 1993; Rao et al., 1998; Gill and Burke, 1999; Jackson et al., 2000; Jobba´gy and Jackson, 2000; Ong et al., 2001; Jose, 2009; Nair, 2009). Commonly used tree species can include native species and those ideal for a specific role. For example, *Gliricidia sepium* (Jacq.) Welp. is often used as live fences, *Pseudosamanea guachapele* (Kunth) Harm. is often used as fodder and *Enterolobium cyclocarpum* (Jacq.) Griseb. is often used as shade and for forage in Costa Rica (Jesus, 2008). However, in order for this practice to remain effective, both environmentally and economically, the prominent species used must be resilient to possible future changes in climate including changes in temperature and atmospheric concentrations of carbon dioxide (CO_2).

It is possible for CO_2 to impact the growth of vegetation, including trees, with a fertilization effect through the acceleration of photosynthesis rates, hence increasing growth and biomass accumulation. The CO_2 fertilization effect is a function of stomatal conductance, where higher concentrations of CO_2 allows for a more narrow opening of the stomata thereby conserving water and decreasing respiration resulting in greater water use efficiency (Korner, 2000; Notoro et al., 2007).

Within the tropical setting the ability of leaves to respond to elevated CO_2 depends on the presence and conditions of other variables including nutrient availability. When there is the capacity for carbohydrate formation then the response is an increase in the rate of

photosynthesis. However, if an excess amount of CO_2 is absorbed into the leaf than can be efficiently utilized than the rate of photosynthesis can decline (Chambers & Silver, 2004). In a study by Lovelock et al. (1999) *Luehea seemanii* Planch. & Triana branchlets were enriched with CO_2 within a tropical forest in Panama and demonstrated an increase in photosynthetic carbon (C) fixation and a decrease in stomatal conductance. However, it did not lead to an increase in biomass within the leaves, the occurrence of flowers or fruit or the concentration of non-structural carbohydrates within the leaves. There was an increase in non-structural carbohydrates within the woody stem tissue indicating that the storage of the increased fixed C has the potential to be in long term sinks such as the woody material rather than high turnover biomass such as roots and leaves. Other field experiments with tree seedlings, for example *Beilschmiedia pendula* (Sw.) Hemsl., and *Tachigalia versicolor* Standl. & L.O. Wms. trees grown in Panama, exposed to elevated CO_2 have shown variable responses depending on the species involved and when there was an increase in the growth rate it diminished over the long term (Lovelock et al., 1998; Wurth et al., 1998; Clark, 2004; Cramer et al., 2004).

Photosynthesis is the most heat sensitive process undergone by plants (Chambers & Silver, 2004). Photosynthesis has a parabolic relationship with temperature; up to an optimum level there is an increase in photosynthetic levels, however beyond that rapid decline can occur (Clark, 2004). It was found that the threshold level in tropical forest species is approximately greater than or equal to 30ºC at the leaf surface and this level has been reached and exceeded during at least half of the year from 1995-1998 (Keller & Lerdau, 1999). The effect of temperature alone by raising respiration rates and decreasing photosynthesis could lead to an overall decrease in net primary production (NPP) and a decline in the potential to store C (Keller & Lerdau, 1999).

The combined impact of the rise in temperature and CO_2 has some variable responses on vegetation. The CO_2 fertilization effect is believed to vary with differing temperatures. As temperature increases the oxygenation reaction catalyzed by *Rubisco* has a competitive advantage over the carboxylation reaction catalyzed by the same enzyme (Chambers & Silver, 2004).

Overall, while there has been thorough investigation into how trees in the tropics may react to the individual variables involved in climate change (Lovelock et al., 1998; Keller & Lerdau, 1999; Chambers & Silver, 2004) there is still no certainty on how the interactive effects will impact the trees (Chambers & Silver, 2004; Clark, 2004). In addition, it was acknowledged in numerous studies that the effects were variable depending on the species type and very limited research has concentrated on the study of agroforestry tree species (Keller & Lerdau, 1999; Chambers & Silver, 2004; Clark, 2004). Therefore, while general responses can be inferred no direct evidence is currently available to provide insight into how these specific tree species may react to the changing climate.

The specific objectives of this study were:

1. To quantify the effect of elevated CO_2 (800 ppm), temperature (+2°C daytime and +3°C nighttime) and a combined treatment (CO_2 and temperature) on the growth and survival of *Cedrela odorata* L. and *Gliricidia sepium* (Jacq.) Walp, seedlings.

2. To quantify changes in *C. odorata* and *G. sepium* leaf C and N concentration between treatments; elevated CO_2 (800 ppm), temperature (+2°C daytime and +3°C nighttime) and a combined treatment (CO_2 and temperature).

4.2 Materials and Methods

4.2.1 Selection of Experimental Tree Species

Gliricidia sepium is naturally found in a range from Mexico, throughout Central America and Columbia however it has now been introduced throughout to other locations within the tropical and subtropical biomes (Joker, 2002). Originally, this tree grew in tropical deciduous forests, on hillsides and ravines (Csurhes and Edwards, 1998). This fast growing tree is able to flourish at various altitudes, areas with rainfall as low as 400 mm/year but grows best with over 900 mm/year, and will grow in soils from heavy clays to sands and on rocky eroded sites (Csurhes and Edwards, 1998). Water logging and acidic conditions causing aluminum saturation are the two known conditions which *G. sepium* is highly sensitive to (Suittie, 2005). *Gliricidia sepium* typically attains 10 to 12 m in height and basal diameter reaching 50-70 cm. Leaves are once-pinnate, usually alternate, positioned subopposite or opposite and reach approximately 30 cm long. The leaves are composed of six to twenty four leaflets that are oval or elliptical in shape. Leaflets tend to be two to eight cm long and one to three cm wide (Suittie, 2005; CATIE, 1991).

Due to the following characteristics, *G. sepium* is representative of a widely used multipurpose agroforestry tree (Suittie, 2005). It is a N_2-fixing species, having a symbiotic relationship with *Rhizobia* bacteria, with a high nutrient content in the leaves, making it suitable for mulch and green manure (Joker, 2002). As well, its ability to re-sprout repeatedly after intensive pruning allows for high biomass utilization and control of the extent of shading at any point in the year (Joker, 2002). These trees are often utilized as living fences and windbreaks in silvopastoral applications and can also be used for timber, fuel and furniture production (Duke, 1983).

Cedrela odorata grows naturally from Mexico, throughout Central America and to the Northern areas of Argentina (Cavers et al., 2004; Cintron, 2005). Typically, *C. odorata* is found in mixed semi-deciduous or semi-evergreen forests (Cintron, 2005). These trees are able to grow in areas with dry seasons and a precipitation range of 1200-2400 mm (Cintron, 2005). This tree species is often found over limestone clay soils or volcanic origin soils with a tolerance for acidic conditions (Cintron, 2005). *Cedrela odorata* can reach 40 m in height and 120 cm in diameter (Cavers et al. 2004). Leaves are paripinnate or imparipinnate, 15 to 50 cm long and are made up of 10 to 22 oblong or lancelet leaflets (Rocas, 2002). *Cedrela odorata* is a fast growing tree species, often sought after for its wood for furniture production and other value added products (Rocas, 2002). It is resistant to many insects and fungus thereby making it selected by farmers. It is often used as a windbreak or riparian barrier in silvopastoral systems (Rocas, 2002; Ricker et al., 2000).

Both *G. sepium* and *C. odorata* shared common characteristics which made them ideal for this experiment. Both species had seeds which remained viable over a long-term and which germinated quickly (Cintron, 2005; Suittie, 2005). In addition, both species were fast growing at the seedling stage allowing for some differences in height to be seen over the five week growing period (Cintron, 2005; Suittie, 2005). *Gliricidia sepium* represents a widely used species in Costa Rica for the environmental benefits such as N_2-fixing and the high nutrient content in the leaf and biomass (Suittie, 2005). On the other hand *C. odorata* is not as widely used and when used it's a means for protection from insects or a value added product of lumber (Rocas, 2002). Therefore, each species represents a different need which trees can fill in an agroforestry setting.

4.2.2 Tree Growth Conditions

In order to determine relevant changes in growth within the context of tropical silvopastoral systems it was necessary to identify the most sensitive and economically important life stage of growth. In this case the early seedling stage, when the trees are first planted and rapid successful establishment is essential, is the most relevant. For the purposes of this research it was decided that a five week growth experiment following a controlled germination period would provide sufficient insight into changes in seedling establishment between treatments.

Seeds of both species were attained from the forest seed bank at the Centro Agronómico Tropical de Investigacíón y Enseñanza (CATIE), Costa Rica in August of 2008. Germination of the seeds was the same for all treatments and followed the procedure which accompanied the seeds when purchased. The method was similar between species. For both species twenty seeds were germinated, ten per Petri dish. The seeds were submerged in room temperature water (21°C) and kept in darkness. The seeds were removed from the water after 12 hours for *C. odorata* and after 24 hours for *G. sepium* and covered in moist sand for the remainder of the germination process. The sand was kept moistened throughout and the Petri dishes were kept in a darkened state. On average, germination was completed within two weeks for *G. sepium* and three weeks for the *C. odorata*. The visual indicators of a successful germination were the formation of root structures, the opening of the seed covering and the beginning of cotyledon separation (Evans, 1972). The eight most successfully germinated seeds were chosen for planting.

Changes in seedling growth rates were determined by the use of controlled environmental chambers (PGR15, Conviron Canada). The use of controlled environmental chambers is ideal for this study because of its precise control of various climate variables

including temperature, humidity, light intensity and CO_2. Controlled environmental chambers supply the only current means in climate change research to manipulate both variables at the same time. These results will provide insight into the response of the seedlings to different temperatures and CO_2. It should be noted that environmental chambers do not mimic all field conditions such as the level of light intensity found in the field, the presence of pests, wind and competition. Therefore, the results will not be presented as a reflection of tree seedling growth in the field, but will provide insight into the response of tree seedlings to changes in climate during their initial growth phase. Within this experiment tree seedlings were grown under four different treatments:

1. Ambient conditions (360 ppm for concentration of CO_2 and 32º C as mean daytime temperature, 22 ºC as mean nighttime temperature), from here on referred to as AMB

2. Elevated concentrations of CO_2 predicted for the year 2100 (800 ppm CO_2 and 32º C and 22º C), from here on referred to as CO2

3. Elevated temperature predicted for the year 2100 (360 ppm CO_2 and 34ºC and 25ºC), from here on referred to as TEMP

4. Combination of elevated CO_2 and temperature (800 ppm CO_2 and 34ºC and 25ºC), from here on referred to as TEMPxCO2

The values chosen are a reflection of the UK Hadley Centre Global Climate Model (GCM) predictions used in the Intergovernmental Panel for Climate Change (IPCC) reports (2007). These predictions are based on the A2 scenario which assumes a slow continuous increase in human population, steady regional economic growth and fragmented technological advancements (IPCC, 2007). This scenario presents predictions that are not the most extreme but still reflect a more pessimistic future global outlook.

All treatments had eight tree seedling replications for each species. All other conditions such as daylight hours, humidity etc. were consistent throughout the treatments

and reflected conditions found in Esparza, Costa Rica. Light intensity was as closely mimicked to the field conditions as could be done with controlled environmental chambers (Appendix 1). Daily variations in temperature however do occur in the field and are not accounted for in the controlled environmental chambers. In these experiments where controlled environmental chambers were used it was the most practical to maintain a constant elevated temperature rather than attempting to mimic daily variability. The schedule of conditions for each treatment used in the controlled environmental chamber can be found in Appendix 1.

Tree seedlings were planted in pots with measurements of 20.3 cm diameter and 14 cm height. This provided the minimum rooting area necessary for growth. The potting soil was a general purpose soil (Premier Horticulture, Pro-mix Bx Mycorise Pro, Canada) with mycorrhizal inoculum (*Glomus intraradices*). The soil was derived from Canadian sphagnum peat moss (75-85%/volume), horticultural grade perlite and vermiculite and dolomitic and calcitic limestone. Based on 900 mm of average rainfall per year for the specific region studied, 120 ml of water was delivered every two days to the trees. Although this geographical area, as discussed previously, experiences seasonal extremes in rainfall, for the purpose of this research it was determined, through discussions with the local farmers, that mean levels of precipitation would be the most appropriate to reflect typical conditions when farmers plant trees on their fields. Once a week the tree order within the chamber was randomly changed using the random number generator in order to compensate for any condition variability within the chamber (Potvin et al., 1990). The schematic of the tree positions within the chamber and the weekly order for each treatment are outlined in Appendix 2. At day 21, a general fertilizer (Schultz Liquid Plant Food, 10-15-10) was applied to all trees by the addition of four drops to every litre of water used for all treatments in order to supplement the potting soil used.

4.2.3 Quantification of Tree Seedling Growth

Observations on tree height, leaf number, tree color and overall health were taken every two days throughout the five weeks of growth when watering occurred. Tree height was determined by placing the ruler at the marked ridge on the pot in order to avoid fluctuations due to soil unevenness and eventual compaction. Leaf number included buds of new leaf growth and did not include leaves that may have died throughout the five week growth phase. Tree color was assessed using qualitative observations every two days for the five week growing period.

Leaf surface area was measured one day before destructive sampling of the trees occured. This measurement was determined by tracing each leaf onto grid paper, cutting out the shapes and analyzing the shapes with the LI-COR (LI-3000) portable area meter to determine the total area for each tree at the end of the five week period. While other direct surface area measurements techniques exist such as the graphical method, where the squares are manually counted, the accuracy and variability of the applied method was comparable (Beerling and Fry, 1990).

Once the five week growth period was completed the trees were removed from the environmental chamber. After removal, each tree was extracted from the pot and the soil was separated from the roots. This was first done by physically breaking up the soil and shaking it off the roots. When most of the soil was removed the roots were soaked in water in order to remove attached debris. Although the goal was to remove all soil particulate matter from the roots, it is possible that not all of the soil was removed or that some of the roots were removed accidently with the debris. Once manually dried with paper towel, the tree was weighed (wet weight). Subsequently the trees were dried at 80 °C for 48 hours and were weighed (dry weight) (Evans, 1972). The dried root and shoot were separated and weighed

in order to attain a root:shoot ratio. Leaf area ratio was derived from dividing leaf area by the final dry weight of the plant.

a) 

b) 

Figure 4.1: a) A *G. sepium* seedling at the end of the 5 week growth period b) The root system of the *G. sepium* when removed from the pot at the end of the 5 week growth period

4.2.4 Quantification of Leaf Nutrient Concentration

The chosen trees represented the smallest, medium and tallest trees for each species within each treatment. Leaf samples were dried at 65°C for 36 hours and then ground with a Kinematica Polymix plant grinder (Px-MFC 90D, NY) using a 2 mm sieve and then a Retsch Ball Mill (MM 200, PA). The samples were analyzed for C and N concentration using a Costech Elemental Analyzer (ECS 4010, CA) though elemental combustion analysis.

4.2.5 Statistical Analysis

All data were examined for homogeneity of variance using the Levene Test and normal distribution was tested using the Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test. If the data did not meet the Levene Test it was natural log transformed in order to meet the assumptions (Evans, 1972; Zar, 1984). All of the growth variables were compared between treatments using a one-way analysis of variance (ANOVA) using SPSS (SPSS Science Inc. 2009). Significant differences were tested using the Tukey's least significant difference multiple comparison test (Zar, 1984). Tree height data were corrected by subtracting the first days measurement from all of the subsequent days measurements in order to quantify the change in growth over the five week period. For all statistical analyses, the threshold probability level for determining significant differences was $p<0.05$.

4.3 Results

4.3.1 Tree Seedling Growth Measurements

For *G. sepium* the CO2 and TEMPxCO2 treatment both had 100% survival, followed by the TEMP treatment with 86% survival and the AMB treatment with 75% survival. For *C. odorata* the TEMP treatment had 100% survival, followed by the AMB and TEMPxCO2 treatments both with 86% survival and the CO2 treatment with 50% survival. The overall growth rate for all the *C. odorata* trees followed a similar trend of a positive generally curvilinear growth (Fig. 4.3). *Gliricidia sepium* demonstrated a more positive linear growth rate (Fig. 4.4). For *C. odorata* the final height for the TEMPxCO2 treatment was significantly greater than all the other treatments. The CO2 treatment had a final height that was significantly lower than the TEMPxCO2 and TEMP treatment. For *G. sepium* the TEMPxCO2 treatment and TEMP treatment were significantly higher in the final height than AMB and CO2 (Table 4.1).

Mean leaf number for *C. odorata* followed a trend of AMB> TEMPxCO2> TEMP> CO2. For *G. sepium* the trend was AMB> TEMP> TEMPxCO2> CO2 (Fig. 4.5). Leaf number for both species did not pass the Levene test and for that reason the values were log transformed in order to do the analysis. Tree leaf number, when log transformed, demonstrated significant differences between treatments. For *G. sepium,* the TEMP and TEMPxCO2 treatment were significantly greater than AMB and CO2 (Table 4.1). For *C. odorata* the AMB and TEMPxCO2 treatment were significantly greater than the CO2 treatment. However, no significance was found between the TEMP treatment and any of the other treatments (Table 4.2)

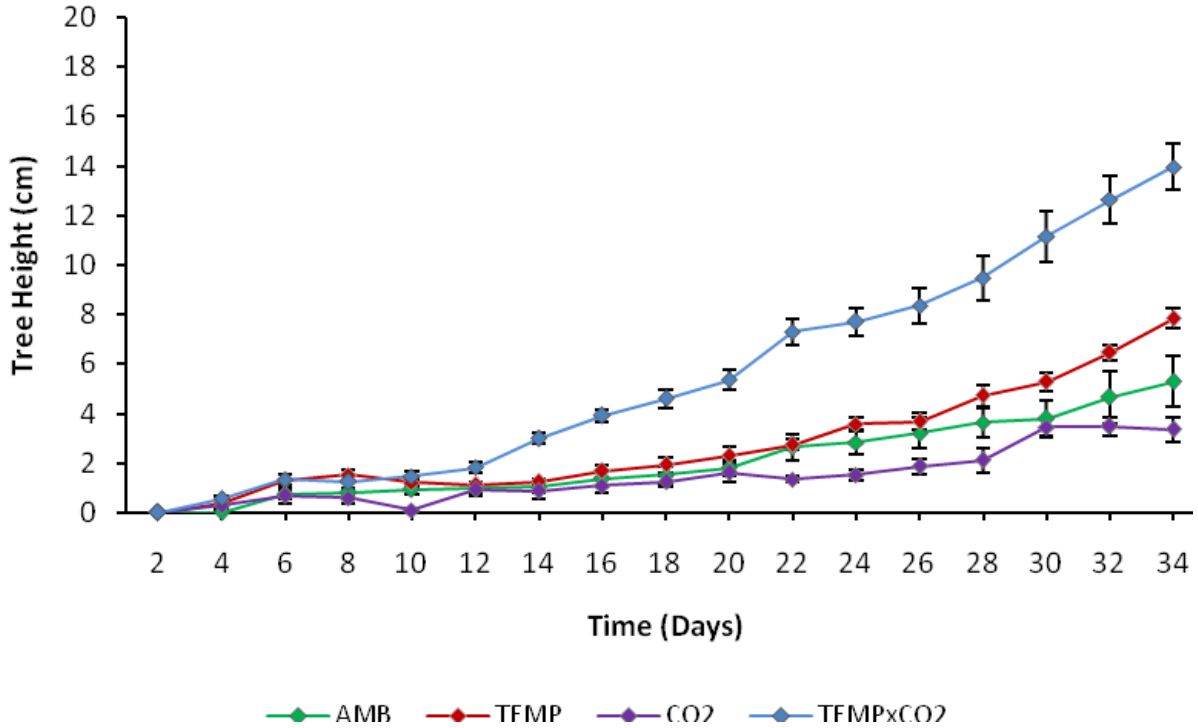

Figure 4.2: Mean *C. odorata* height (cm) under the four treatments; ambient (AMB: 360 ppm CO_2, 32° C daytime and 22° C night time temperature), CO2 treatment (CO2: 800 ppm), temperature treatment (TEMP: 34° C daytime and 25° C night time temperature) and combined treatment (TEMPxCO2: 800 ppm, 34° C and 25° C) over the five weeks of growth. Standard error bars shown for all data points.

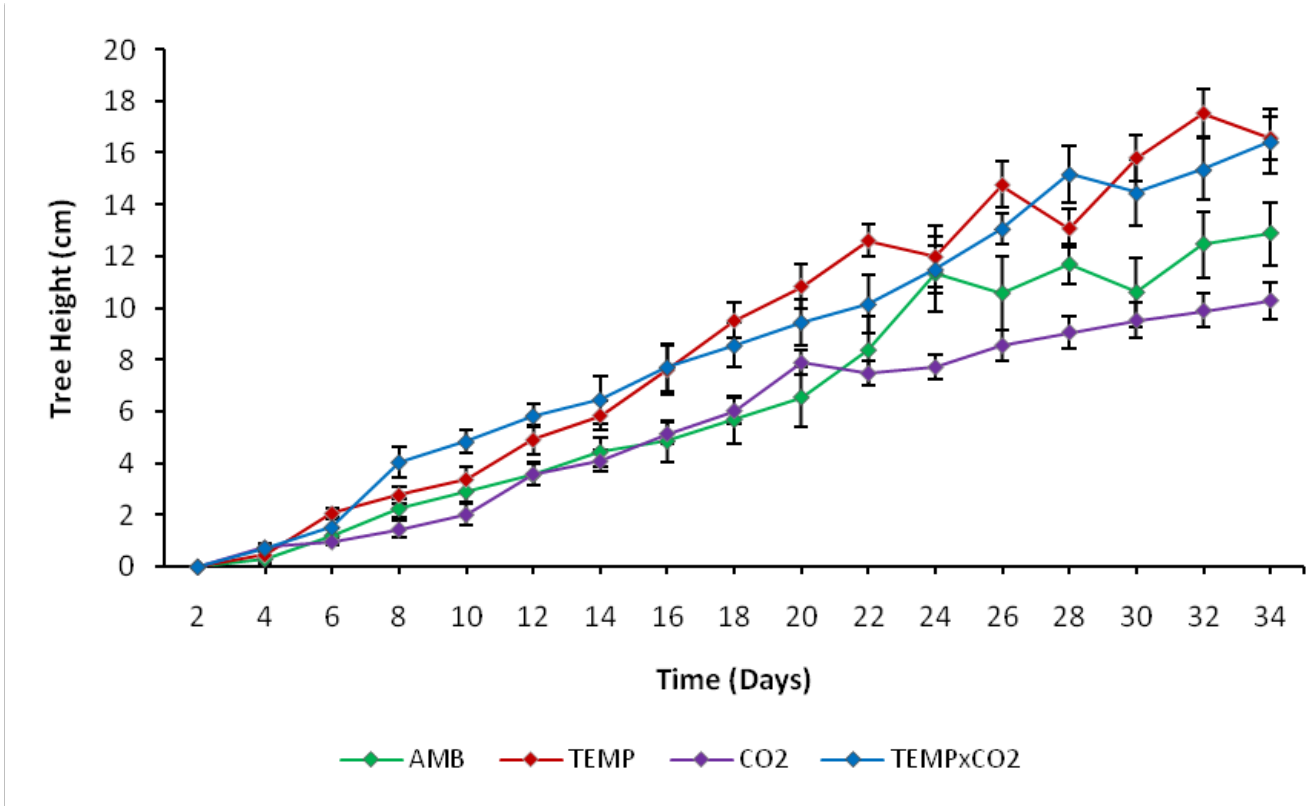

Figure 4.3: Mean *G. sepium* height (cm) under the four treatments; ambient (AMB: 360 ppm CO_2, 32° C daytime and 22° C night time temperature), CO_2 treatment (CO2: 800 ppm), temperature treatment (TEMP: 34° C daytime and 25° C night time temperature) and combined treatment (TEMPxCO2: 800 ppm, 34° C and 25° C) over the five weeks of growth. Standard error bars shown for all data points.

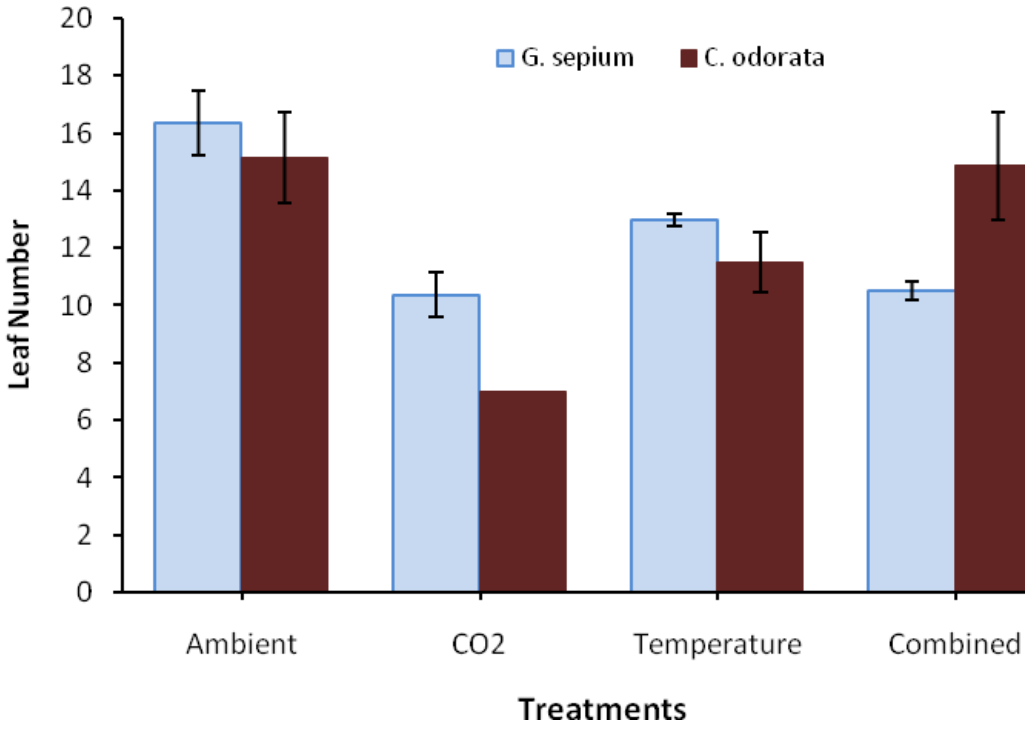

Figure 4.4: Comparison of mean leaf number between *G. sepium* and *C. odorata* for all treatments; ambient (AMB: 360 ppm CO_2, 32° C daytime and 22° C night time temperature), CO_2 treatment (CO2: 800 ppm), temperature treatment (TEMP: 34° C daytime and 25° C night time temperature) and combined treatment (TEMPxCO2: 800 ppm, 34° C and 25° C). Standard error bars included.

Table 4.1 Summary of mean growth variables and significance between treatments; ambient (AMB: 360 ppm CO_2, 32° C daytime and 22° C night time temperature), CO_2 treatment (CO2: 800 ppm), temperature treatment (TEMP: 35° C daytime and 26° C night time temperature) and combined treatment (TEMPxCO2: 800 ppm, 34° C and 25° C) results for *G. sepium*, standard error is indicated in parentheses.

Treatments	*Final Tree Height (cm)*	*Leaf number (log)*	*% water*	*Root:shoot ratio*	*Leaf area ratio*
AMB	12.88 (1.28) [a]	2.53 (0.10) [a]	84.76 (0.46) [a]	0.66 (0.05) [a]	95.02 (6.65) [a]
TEMP	16.59 (0.89) [b]	2.80 (0.06) [b]	83.72 (0.52) [a]	1.06 (0.10) [a]	80.33 (6.05) [a]
CO2	10.30 (0.71) [a]	2.32 (0.07) [a]	83.41 (0.59) [a]	1.44 (0.15) [b]	59.09 (3.79) [b]
TEMPxCO2	16.44 (1.25) [b]	2.78 (0.08) [b]	82.44 (0.43) [b]	1.54 (0.10) [b]	56.60 (2.19) [b]

Note: Significant differences between treatments ($p < 0.05$) are indicated by differing lower case letters.

Table 4.2: Summary of mean growth variables for *C. odorata*. Significance between treatments; (AMB: 360 ppm CO_2, 32° C daytime and 22° C night time temperature), CO_2 treatment (CO2: 800 ppm), temperature treatment (TEMP: 35° C daytime and 26° C night time temperature) and combined treatment (TEMPxCO2: 800 ppm, 34° C and 25° C) indicated. Standard error is indicated in parentheses.

Treatments	*Final Tree Height (cm)*	*Leaf number (log)*	*% water*	*Root:shoot ratio*	*Leaf area ratio*
AMB	5.83 (1.03) [a]	2.68 (0.32) [a]	86.61 (1.15) [a]	0.48 (0.04) [a]	153.27 (16.09) [a]
TEMP	7.84 (0.39) [a]	2.41 (0.27) [a]	86.45 (0.51) [a]	0.98 (0.07) [b]	143.63 (11.16) [a]
CO2	3.35 (0.50) [a]	1.95 (0.00) [b]	84.95 (0.92) [a]	1.24 (0.20) [b]	121.06 (15.51) [a]
TEMPxCO2	13.96 (0.92) [b]	2.65 (0.34) [a]	85.13 (0.31) [a]	1.14 (0.09) [b]	112.01 (11.13) [a]

Note: Significant differences between treatments ($p < 0.05$) are indicated by differing lower case letters.

Percent water in the tree seedlings was not significantly different for *C. odorata* between treatments; however for *G. sepium* the TEMPxCO2 treatment had significantly lower percent water than the AMB treatment. The root:shoot ratio for *G. sepium* was significantly greater for the CO2 and TEMPxCO2 treatment (Table 4.1). For *C. odorata* all the treatments were significantly greater than the AMB treatment (Table 4.2). The leaf area ratio for *G. sepium* was significantly lower for the CO2 and TEMPxCO2 treatment. For *C. odorata* no significant difference was found.

4.3.2 Leaf Nutrient Concentration

The leaf nutrient concentration for *C. odorata* demonstrated a significantly lower N concentration for the CO2 treatment and this caused a significant increase in the C:N ratio (Table 4.3). For *G. sepium* the CO2 treatment and TEMPxCO2 treatment had significantly lower N concentration and for both of these treatments the C:N ratio reflected this with a significant increase (Table 4.3).

4.3.3 General Observations

Plant leaf color and general observations changed between treatments and species. *Cedrela odorata* predominantly had brown leaf tips in the older leaves by the end of the five weeks of growth. This was especially pronounced in the TEMPxCO2 treatment where every tree had at least two brown leaf tips. In addition, *C. odorata* demonstrated the beginning stages of lignification in the stem which was especially pronounced for the TEMP and TEMPxCO2 treatments. For *G. sepium* the leaves maintained a green color for all the treatments; however both the TEMP and TEMPxCO2 treatments displayed light spotting on older leaves. This species also demonstrated the beginning stages of lignifications, bark

formation, in the stem which was most pronounced in the TEMP and TEMPxCO2 treatments.

Table 4.3: Summary of C, N concentration and C:N ratio for the leaves of *C. odorata* and *G. sepium* under the treatments (AMB: 360 ppm CO_2, 32° C daytime and 22° C night time temperature), CO_2 treatment (CO2: 800 ppm), temperature treatment (TEMP: 35° C daytime and 26° C night time temperature) and combined treatment (TEMPxCO2: 800 ppm, 34° C and 25° C). Standard error is represented in parentheses.

Species	*Treatment*	*Carbon (g/kg)*	*Nitrogen (g/kg)*	*C:N ratio*
C. odorata	AMB	437.8 (4.96) [a]	32.0 (0.41) [a]	13.67 (0.06) [a]
	CO2	436.6 (2.17) [a]	12.1 (0.33) [b]	36.23 (0.83) [b]
	TEMP	450.6 (6.12) [a]	35.3 (1.24) [a]	12.78 (0.43) [a]
	TEMPxCO2	445.7 (0.87) [a]	31.6 (2.54) [a]	14.27 (1.12) [a]
G. sepium	AMB	437.8 (4.96) [a]	23.9 (2.57) [a]	18.75 (2.13) [a]
	CO2	436.6 (2.18) [a]	11.5 (0.27) [b]	37.90 (0.99) [b]
	TEMP	437.3 (2.34) [a]	21.1 (0.46) [a]	20.71 (0.35) [a]
	TEMPxCO2	423.9 (3.89) [a]	12.5 (0.88) [b]	34.10 (1.96) [b]

Note: Significant differences between treatments ($p < 0.05$) are indicated by differing lower case letters.

4.4 Discussion

4.4.1 Tree Seedling growth measurement

As was hypothesized, growth response to different climatic conditions varied by species. Seedling survival rate may indicate that any change in climate would be beneficial for *G. sepium*. For *C. odorata* survival indicates that the best conditions would be a warmer climate but not one with elevated CO_2. However, due to the small number of seedlings in each treatment it is difficult to make any generalizations. Typically, survival is a function of seedling size, rate of growth and competition for and availability of resources (Collet and Le Moguedec, 2007). These factors often reflect success in germination and the growing conditions, so no one variable can be the direct cause for survival. For example genetic variability within each species may have caused differences in seed size and shape which could influence the survival of seedlings past the germination stage (Khurana and Singh, 2001).

Although tree height is not correlated with biomass, it is still a factor necessary for the survival and successful establishment of agroforestry tree seedlings (Nair, 1993). Rapid early seedling growth is an indicator valued by farmers of successful establishment and a characteristic of both species. For example, Turner (1990) demonstrated, in a field study measuring the survival of tree seedlings in the Malaysian tropical rain forest, that seedling height was inversely correlated with mortality. For both species the TEMPxCO2 treatment demonstrated the greatest significant overall increase in height. As well, for *G. sepium* the TEMP treatment resulted in a significant increase in total height. These findings are supported by a study in Martin et al. (1995) and Allen and Vu (2008) where an increase in temperature and CO_2 (+4.5°C and 700 ppm CO_2) increased the growth of citrus trees (*Citrus reticulata* B., *Citrus limon* (L.) Burm.) in Gainesville, Florida. However, unlike previous

studies where a fertilization effect was clearly demonstrated (Wurth et al., 1998; Raizada et al., 2009; Lindroth, 2010) there was no significant CO_2 fertilization effect for either species, for any growth indicator. *Gliricidia sepium* had a greater growth under the higher TEMP as compared to the AMB and CO2 treatment which could be a reflection of its natural optimal temperature requirements. The results of the TEMPxCO2 treatment could be reflecting these optimal temperature requirements (Khurana and Singh, 2001). In a similar study by Overdieck et al. (2007) where European Beech (*Fagus sylvatica* L.) saplings were grown in field phytotron chambers, they also found elevated temperature (+4 °C) resulted in greater tree height and stem biomass. This study also demonstrated greater sapling height for the CO2 treatment (700 ppm) and TEMPxCO2 treatment (+ 4°C and 700 ppm) (Overdieck et al., 2007).

For *G. sepium* leaf number was a reflection of tree height and could be attributed to the same influences as differences in height. Leaf number was significantly lower for the CO2 treatment of *C. odorata*, however this could be an artefact of the high mortality rate. While it is not common for growth to decrease under elevated CO_2 conditions, it has been shown that if an excess of CO_2 is taken up than can be utilized, a decrease in photosynthesis may occur (Chambers and Silver, 2004). However, other factors may contribute to the loss of photosynthesis. For example, a study by Thomas and Strain (1991) demonstrated that the transfer of cotton plants (*Gossypium hirsutum* L.) from a small pot to a large pot decreased the effect of root stress allowing for the expression of CO_2 fertilization. As well, the lack of bioavailable N or other nutrients such as phosphorus can cause the same effect (Hsiao and Jackson, 1999). If this was the case, it would be the most pronounced in *C. odorata*, because *G. sepium* is an N_2-fixing species and would therefore not be limited by the availability of N (Suittie, 2005).

It was expected that no change in percent water would occur since it is a reflection of the structural capacity of the plant and this study is comparing seedlings that are the same age and under the same water treatment (Evans, 1972). However, since no study or information pertaining to a significant difference in water content can be found any difference in percent water could be a reflection of experimental error. Either, not enough water was administered the day before extraction, the oven conditions were not consistent with the other treatments or the dried tree was allowed to reabsorb atmospheric moisture when removed from the oven.

The root:shoot ratio was significantly higher in the case of *G. sepium* for the CO2 and TEMPxCO2 treatment. For *C. odorata* it was significantly higher for all the treatments compared to the AMB conditions. This is quite different from other studies which demonstrated a decrease in root:shoot ratio when exposed to elevated CO_2 (Overdieck et al., 2007;Allen and Vu, 2008). These studies demonstrated that most growth occurred above ground under the differing temperatures (+4 °C and +4.5 °C) and a CO_2 concentration of (700 ppm). This is supported by commonly accepted plant physiology theory where factors which improve growing conditions often lead to reduced root:shoot ratios (Evans, 1972; Harris, 1992). In a study by Markesteijn and Poorter (2009) 62 tropical dry and moist forest seedlings in Bolivia were grown under various light treatments (shade and open canopy) and water treatments (drought and moist conditions). This study demonstrated that tree growth occurs to maximize the most limiting necessary component for growth when under stress (Markesteijn and Poorter, 2009). When taken in the context of this study, the increase in root:shoot ratio is a reflection of the optimum above ground conditions resulting in a greater requirement for belowground resources in order to maintain high rates of photosynthesis. As a result of an increase in root mass, an increase in soil fertility in the area surrounding the tree due to root turnover could occur. For *G. sepium* this is further emphasized by the associated significant decrease in leaf area ratio for the CO2 and TEMPxCO2 treatments reflecting the

possible allocation of plant biomass in the root mass. The lack of significant change in leaf area ratio for *C. odorata* indicates that although the treatments have an increase in rooting mass it has not reduced the photosynthetic potential of the seedlings. Other studies, indicated an increase in leaf area and leaf area ratio under elevated CO_2 conditions which correspond to the other findings of a decrease in root:shoot ratios (Khurana and Singh, 2001; Allen and Vu, 2008; Markesteijn and Poorter, 2009).

For both species, the CO2 treatment resulted in significantly lower N concentration leading to an increase in C:N ratio. *Gliricidia sepium* also experienced a decrease in N for the TEMPxCO2 treatment, again leading to a significant increase in C:N ratio. While for *G. sepium* this could be a reflection of the increased root growth leading to a reallocation of nutrient content for root growth, this is not the case in *C. odorata*. It has been demonstrated that N use efficiency increases under CO2 treatments since the N allocated to *Rubisco* can instead be diverted to other metabolic processes (Lindroth, 2010). This effect combined with the increase in carbohydrate production and phenolic concentrations associated with elevated CO_2 could explain the increase in C:N ratio (Lindroth, 2010; Coûteaux et al., 1999). However, in this study there were no significant differences in C leaf concentration between treatments for either species. This indicates that photosynthesis and respiration rates either remained similar between treatments or that rates may have changed but the overall balance remained constant. The individual processes would need to be studied further in order to comment specifically on rates of change. As a result of elevated C:N ratios these leaves have lower nutrient quality and when used as mulch or for any other purpose in order to sustain the fertility of the land they will have a lower rate of decomposition and lower capacity to renew the bioavailable nutrients necessary for crop and forage growth (Ball, 2006).

The ramifications of this study for agroforestry are varied. The response of both trees reflects a species specific response to the different variables in climate change. This

indicates that other tree species used in agroforestry could have similarly varied responses and therefore one model cannot be applied to all of agroforestry. As well, the response of *G. sepium* demonstrates that the benefits which specific species are able to provide presently may diminish in the future. There is also the possibility that other species may become more suited for agroforestry in the future. The short growing phase utilized in this research may help inform how seedlings could react. However, trees utilized for agroforestry remain on the landscape for a longer timeframe than five weeks making it difficult to generalize how adult trees may react to a changing climate. Further studies incorporating longer timeframes could help fill that gap in knowledge and may further contribute to agroforestry practices.

4.5 Conclusions

Under predicted climatic conditions, *G. sepium* and *C. odorata* seedlings reacted very differently. *Gliricidia sepium* seedlings would be best suited for the expected changes in CO_2 and temperature, as proposed by the IPCC, because those conditions would allow for maximum seedling survival rate, height and leaf number. However, if over time this species response of increased root growth and decreased leaf nutrient content continues to further life stages, it could reduce its current use in agroforestry as a source for mulch. *Cedrela odorata,* however; may be more resilient to the overall changes in climate. This study has shown that *C. odorata* may be sensitive to changes in CO_2 alone. It may exhibit a greater growth in height but have no significant change in nutrient concentration if both temperature and CO_2 were to change. However, since *C. odorata* is more commonly used as a barrier for wind or riparian zone changes to leaf nutrient concentration may be of limited importance. Overall, this study demonstrated that species react to differing conditions to varying extents and that understanding the response of these seedlings to the individual variables and TEMPxCO2 treatments can reveal insight into the mechanisms of tree seedling growth. In addition, this

study highlights the importance of understanding the response of economically valued species, such as agroforestry tree species, to climate change. If a change in climate results in altering the valued characteristics of the trees, such as being fast growing or high leaf nutrient quality, then more appropriate species should be selected which will maximize the benefits of agroforestry on a landscape.

Chapter 5: Soil Microbial Community Dynamics and Nutrient Concentration

5.1 Introduction

Agroforestry production is deeply reliant on, and directly impacts, soil constituents including the availability of soil nutrients, soil organic carbon (SOC) and the microbial communities within. While commonly used agroforestry tree seedlings will be impacted by the change in climate, both the microbial communities and the resulting nutrient dynamics will also be affected (Nair, 1993). The soil component of any agricultural system can be divided into physical, chemical and biological characteristics. All characteristics contribute to the fertility or possible productivity of an agroforestry system and can be correlated to the presence of soil organic matter (SOM) (Manlay et al., 2007). The process of decomposition; where non living tissue and constituents are physically and chemically transformed by microbial activity releasing nutrients for use by the trees, crops or grasses found in silvopasture, is one of the main processes contributing to the formation of SOM (Milton and Kaspari, 2007). Soil biota also contribute to nutrient levels through biological nitrogen (N) fixation, where atmospheric forms of N are transformed into bioavailable forms which plants can utilize. As well, soil biota contribute to the physical aggregation of the soil and chemical composition (Lacombe et al., 2009). Therefore; soil biota play an essential role in maintaining the benefits of agroforestry systems including those under a changing climate.

It is well established that an increase in temperature, up to a threshold, results in an increase in enzymatic and microbial activity. This threshold varies between classes of soil microfauna and species. The increase in activity can lead to elevated rates of soil respiration causing the release of carbon dioxide (CO_2) and N mineralization (Raich & Tufekcioglu, 2000; Rustad et al., 2001). Over the long-term; however, elevated temperatures can result in a loss of soil moisture thereby reducing decomposition and N and phosphorus (P) immobilization (van Meeteren et al., 2008).

Climate change is also predicted to lead to elevated levels of CO_2 in the atmosphere. Research to date has demonstrated conflicting responses of soil microorganisms exposed to elevated concentrations of CO_2. Theoretically, elevated concentrations of CO_2 should result in the turnover of greater C rich biomass from roots and tissue into the soil leading to greater biological activity (Drigo et al., 2008). Various free air CO_2 enrichment studies (FACE) demonstrated that no long-term changes in microbial community structure and enzymatic activity occurred (Lipson et al., 2005; Phillips et al., 2002; Sinsabough et al., 2003; Zak et al., 2000). However, other studies noted an increased rate in decomposition and microbial activity (Drissner et al., 2007; Finzi et al., 2006; Marhan et al., 2008). For example, Taneva and Gonzalez-Meler (2008) performed incubation tests on temperate coniferous forest soil to determine changes in soil decomposition rates exposed to 600 ppm of CO_2. The results indicated that CO_2 did increase the rates of decomposition in mineral soil.

Overall, information exists on the impacts of temperature on soil microorganisms but the implications of elevated concentrations of CO_2 are very unclear. While some difficulties exist in testing these effects, including limited methods for field testing, the lack of comparative work is a major gap in knowledge (Pendall & King, 2007). In addition, the lack of research in the tropical setting comparing the capacity of various types of land uses to adapt to possible future changes provides an opportunity for further study (Pendall & King, 2007).

The objectives of this study were therefore:

1) Determine changes in microbial community dynamics of a conventional pasture, silvopastoral system and a 14 year old regenerated forest throughout a 12 week soil incubation exposed to ambient conditions compared to elevated levels of CO_2 (800 ppm).

2) Determine changes in soil carbon and nitrogen concentration of a conventional pasture, silvopastoral system and a 14 year old regenerated forest after a 12 week soil incubation exposed to ambient conditions compared to elevated levels of CO_2 (800 ppm).

5.2 Materials and Methods

5.2.1 Soil Sampling

Soil was sampled from three sites in Esparza, Costa Rica. Each site was visited and sampled on the same day, August 6, 2008. The first site was a 14 year old silvopasture which included a fodder bank and live fencing for the production of dairy. The next site was a 20 year old conventional pasture used for the production of cattle for dairy and meat products. The last site was a 14 year old regenerated forest which was previously degraded pastureland and adjacent to the silvopastoral site. Soil was sampled to a 20 cm depth, using a soil auger. The depth of 20 cm reflects the typical rooting depth and highest soil biological activity zone (Drigo et al. 2008). Three sub-samples were taken for each site and all of the soil was mixed together in order to account for heterogeneity at the sites. At the conventional pasture site and regenerated forest site the three sub-samples were taken following a transect through the site, one sample near one edge, one near the middle and one near the other edge. For the silvopasture site two of the samples were taken randomly in the pasture and one was taken near at fodder bank. A 2.0 kg sub-sample was taken for each site, air dried for seven days and sieved (2 mm) before transport back to Canada.

5.2.2 Soil Incubation Study

Two twelve week incubations occurred; an ambient treatment, from here on referred to as AMB, where conditions reflected the temperature, humidity and concentrations of CO_2 currently occurring in Esparza, Costa Rica and an elevated CO_2 treatment (800 ppm), from here on referred to as CO2. The CO2 treatment condition was based on predictions made from the UK Hadley Centre Global Climate Model (GCM) based on the A2 scenario in the Intergovernmental Panel for Climate Change (IPCC) reports (IPCC, 2007). Both of these two incubations occurred with the use of controlled environmental chambers (PGR 15, Conviron Canada). The specific schedule of conditions for each treatment used in the environmental chambers is outlined in Appendix 1. For

each site, six incubation jars were used per treatment containing 60 g of wet soil. Three jars were destructively sampled at 6 weeks and the rest at 12 weeks for the microbial community analysis and C and N analysis. The jars were covered on all sides to try and prevent exposure to light, however; the tops were left open in order to allow exposure to the differing levels of CO_2. A level of 50% soil water content was maintained for all the soils throughout the incubation by weighing the jars and adding the difference in moisture every two days. As well, the jar arrangement was changed every week in order to compensate for any variability in chamber conditions. The weekly jar order is available in Appendix 3.

Figure 5.1: Incubation jars and configuration within the environmental chamber for the CO_2 treatment after t=6 destructive sampling.

5.2.3 Soil Microbial Community Structure

Microbial community structure was assessed using the Biolog Ecoplates (Biolog Inc., CA, USA) at t=0, t=6 and t=12 for each treatment. The soil from the three jars for each site and time were mixed together into one sample because a single Biolog Ecoplate™ provides a triplicate (n=3). Biolog Ecoplates ™ are based on C substrate utilization causing a change in color development thereby reflecting the presence and activity of differing C utilizing microorganisms (Garland, 1997). A representative 2 g of soil for each site and treatment (AMB and CO2) were taken and placed into a test tube. An amount of 18ml of 0.85% NaCl solution was added to the test tube and then subsequently shaken vigorously for 10 minutes. A serial dilution was performed to reach a 1:10 000 concentration. From this, a 150 µl sample was added into each well of the Ecoplate™ with a multichannel pipette. The Ecoplate™ was incubated at 25 °C and the colour development of each well was read as optical density (OD) at 590 nm with a plate reader at time 0 and every 24 hours thereafter, up to 168 hours. This method is based on the research developed by Garland and Mills (1991) and has been successfully utilized for soil microbial community analysis in many other studies (Gorlenko & Korzhevin, 1994; Zak et al., 1994).

The microbial communities were characterized by several variables. All of the characteristics were determined by using the Ecoplate™ results from day five to seven. Microbial activity in each Ecoplate™ was determined as the average well-color development (AWCD). This was determined by the following equation:

$$AWCD = \sum ODi/31 \qquad (1)$$

Where the value of OD from each of the 31 wells is OD_i.

Microbial richness (R) represents the number of oxidized C substrates (>0.25 OD). The Shannon Index (*H*s) is an indicator of the diversity and evenness of substrate utilization. This was quantified using the following equation:

$$(Hs) = - \sum pi \, (ln \, pi) \qquad (2)$$

Given that *Pi* is the ratio of activity on each substrate *(ODi)* to the sum of activities on all substrates (*ΣODi)* (Garland, 1997). The threshold for a positive test was any positive response above a 0.25 absorbency (Garland, 1997). Optical density data were corrected by blanking each response well against the first well reading (blank well) thereby eliminating absorbance of the C reading and any negative values associated with a control well (Insam and Goberna, 2004; Garland, 1997).

5.2.4 Soil Nutrient Analysis

Soil samples from each treatment and site at t=0, t=6 and t=12 were taken and dried at 50°C for 48 hours. Soil was ground to a fine powder (< 250 μm) using a Retsch Ball Mill (MM 200, PA) and analyzed for soil organic C (SOC) and total N (TN) on a Costech Elemental Analyzer (ECS 4010, CA). Through a hydrochloric acid test it was determined that carbonates were not present. A 2.0 mg sample of soil for each site was put into test tubes and exposed to 3 mL of 3M hydrochloric acid. No effervescence was seen indicating the lack of CO_2 formation.

5.2.5 Statistical Analysis

All data were examined for homogeneity of variance using the Levene Test and normal distribution was tested using the Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test. All of the soil data were compared between sites and treatments using a one-way analysis of variance (ANOVA) using SPSS (SPSS Science Inc. 2009). Significant differences were tested using the Tukey's least significant difference multiple comparison test (Zar, 1984).

Principal components analysis (PCA) was performed on the OD data for the Ecoplate™ C substrate utilization analysis. All of the data met the assumptions of continuous data which is

normally distributed. The Kaiser-Meyer-Olkin measure of sampling adequacy was greater than 0.5 for each individual variable as well as the set of variables. The Bartlett's test of sphericity was significant ($p < 0.05$). Therefore, PCA was the appropriate technique for this data set. For all statistical analyses, the threshold probability level for determining significant differences was $p<0.05$. While other methods of analysis could be applied to this data PCA is the most commonly used and makes these results more comparable to other studies (Garland, 1997).

5.3 Results

5.3.1 Soil microbial community structure dynamics

Results showed that the AWCD is significantly greater for the CO2 treatment compared to the AMB treatment at t=6 for both the pasture and silvopasture sites but no significant differences were found for the regenerated forest site. No significant difference in species richness was observed for any treatment or site. The Shannon index exhibited varied responses. For all sites the t=12 CO_2 treatment demonstrated significantly lower values. For the pasture and regenerated forest site the t=6 AMB treatment also had a significantly lower value (Table 5.1).

The PCA demonstrated components at t=6, t=12 and when all of the times were combined demonstrating trends and relationships in data points. For t=6 the first and second principle components explained 62% and 32% (Fig. 5.2). The AMB treatment for the various sites clustered together and the CO2 treatment for the various sites clustered together. At t=12 the first component explained 69% and the second component explained 16% (Fig 5.3). All of the sites and treatments clustered together except the AMB regenerated forest.

Table 5.1: Average well color development (AWCD), richness (R) and Shannon index (Hs) based on carbon substrate use in Biolog Ecoplate ™ between sites (conventional pasture, silvopasture and regenerated forest soil), sampling time (t=0, t=6, t=12) and treatments (AMB: 360 ppm, CO2: 800 ppm). Standard error given in parentheses (n=3).

Site	*Treatment*	*Time*	*AWCD*	*R*	**Hs**
		t=0	0.67 (0.04)[a]	20 (1.0)[a]	3.10 (0.01)[a]
	AMB	t=6	0.56 (0.03)[a]	18 (1.0)[a]	2.94 (0.01)[b]
Pasture	AMB	t=12	0.52 (0.04)[a]	20 (1.0)[a]	3.07 (0.02)[a]
	CO2	t=6	1.01 (0.21)[b]	21 (1.0)[a]	3.07 (0.03)[a]
	CO2	t=12	0.39 (0.20)[a]	15 (3.0)[a]	2.90 (0.04)[b]
		t=0	0.67 (0.03)[a]	21 (1.0)[a]	3.12 (0.01)[a]
Silvopasture	AMB	t=6	0.60 (0.03)[a]	20 (0.3)[a]	3.03 (0.01)[a]
	AMB	t=12	0.52 (0.06)[a]	22 (1.0)[a]	3.12 (0.02)[a]
	CO2	t=6	1.25 (0.24)[b]	21 (1.0)[a]	3.06 (0.04)[a]
	CO2	t=12	0.35 (0.18)[a]	14 (3.0)[a]	2.94 (0.02)[b]
		t=0	0.66 (0.03)[a]	21 (1.0)[a]	3.10 (0.01)[a]
	AMB	t=6	0.50 (0.02)[a]	18 (0.4)[a]	3.00 (0.01)[b]
Regenerated Forest	AMB	t=12	0.48 (0.01)[a]	19 (0.5)[a]	3.10 (0.01)[a]
	CO2	t=6	0.52 (0.07)[a]	20 (2.0)[a]	3.09 (0.04)[a]
	CO2	t=12	0.36 (0.18)[a]	13 (3.0)[a]	2.96 (0.38)[b]

Note: Significant differences between treatments ($p<0.05$) are indicated by differing lower case letters.

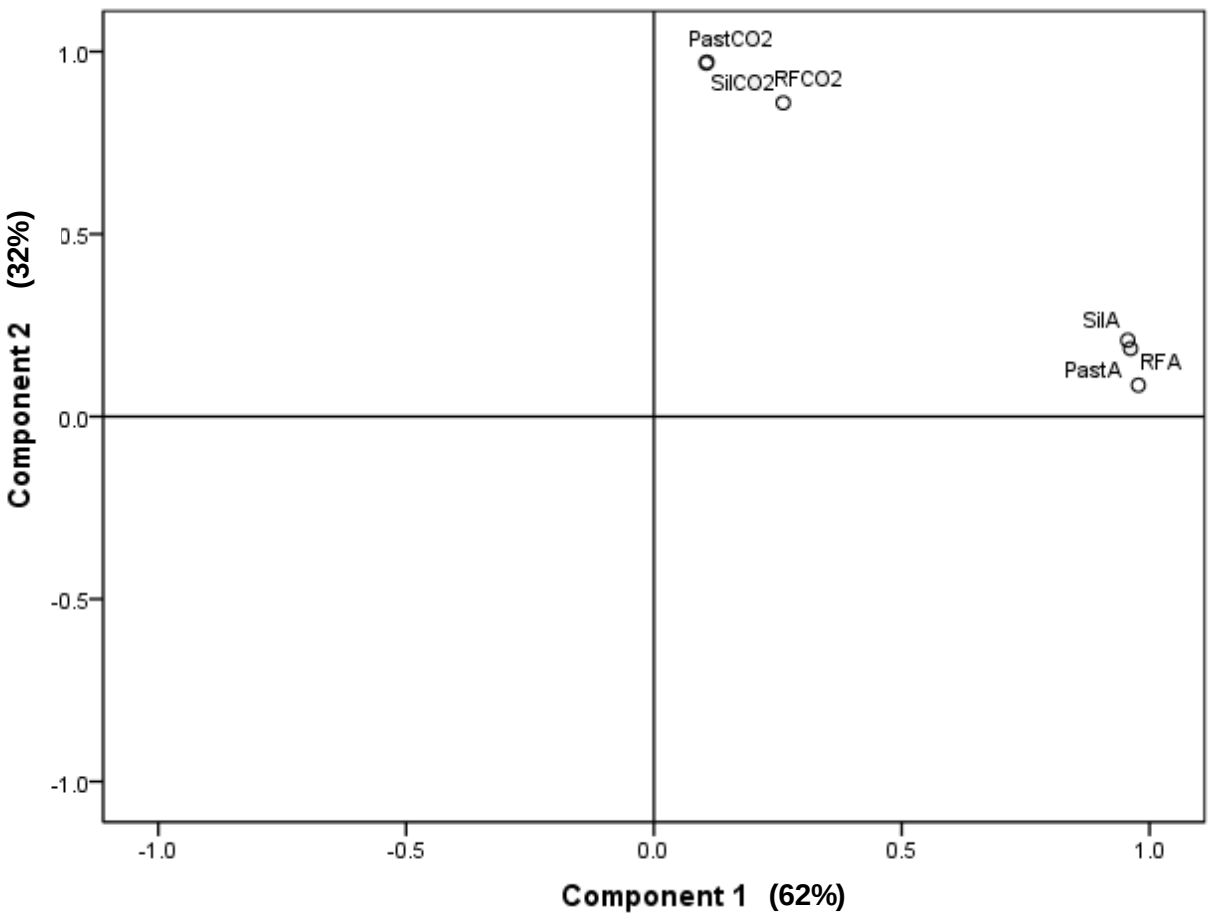

Figure 5.2 Principle components based on carbon source utilization in Biolog Ecoplates™ of conventional pasture (Past), silvopasture (Sil) and regenerated forest (RF) exposed to ambient (A) and elevated CO_2 (800 ppm) conditions at t=6.

When all of the data for each site (pastor, silvopasture and regenerated forest) and treatment (AMB and CO2) were analyzed for principal components three components were found. The first component accounted for 62% of the data, the second component accounted for 17% of the data points and the third for 8% (Fig. 5.4). There were three clusters; the first included all the sites for the AMB treatment at t=12 and t=6, the second included the sites for the CO2 treatment at t=12 and the AMB treatment at t=0, and the last cluster consisted of the sites in the CO2 treatment at t=6 and the regenerated forest site for the AMB treatment t=12 (Fig. 5.4).

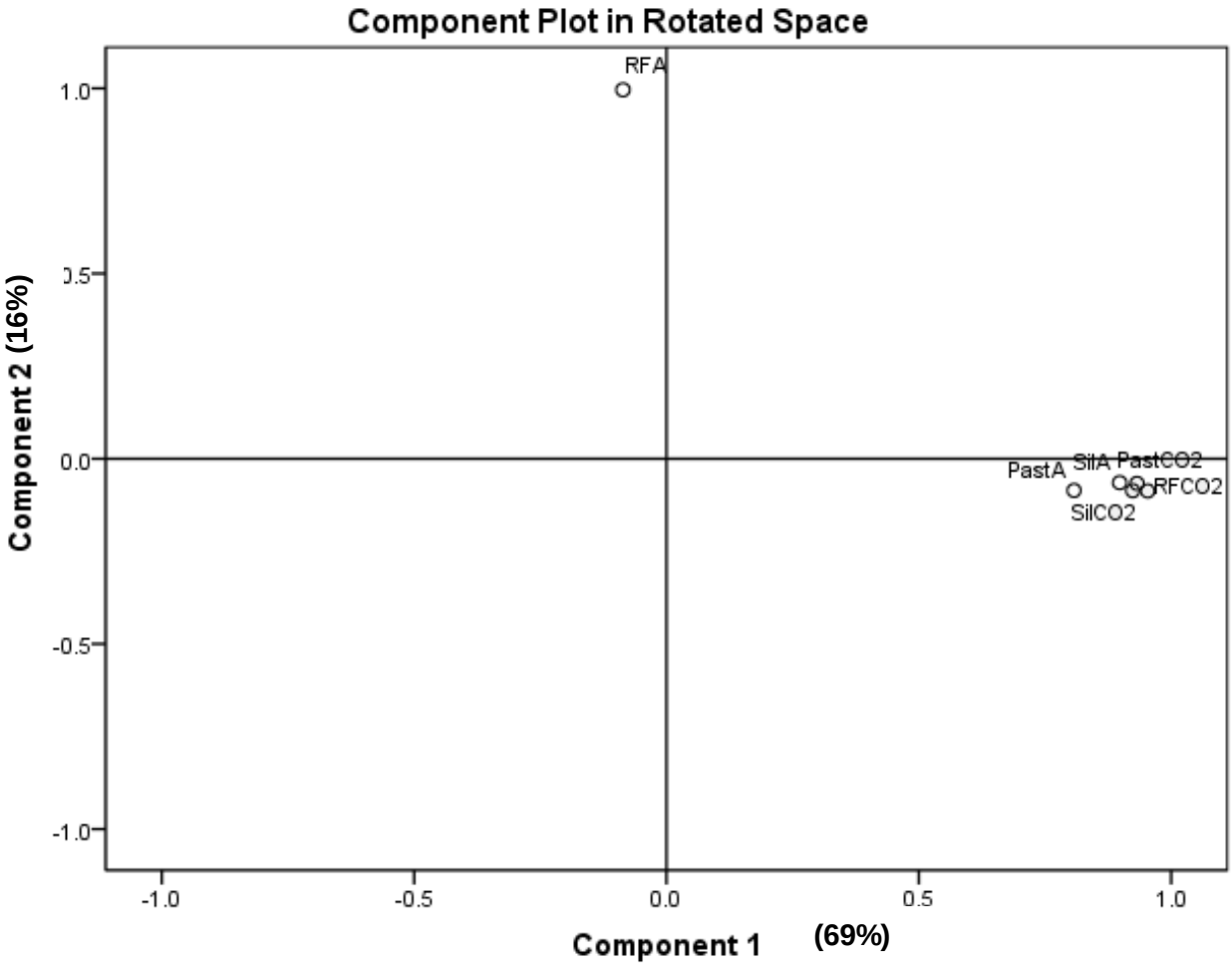

Figure 5.3 Principle components based on carbon source utilization in Biolog Ecoplates™ of conventional pasture (Past), silvopasture (Sil) and regenerated forest (RF) exposed to ambient (A) and elevated CO_2 (800 ppm) conditions at t=12.

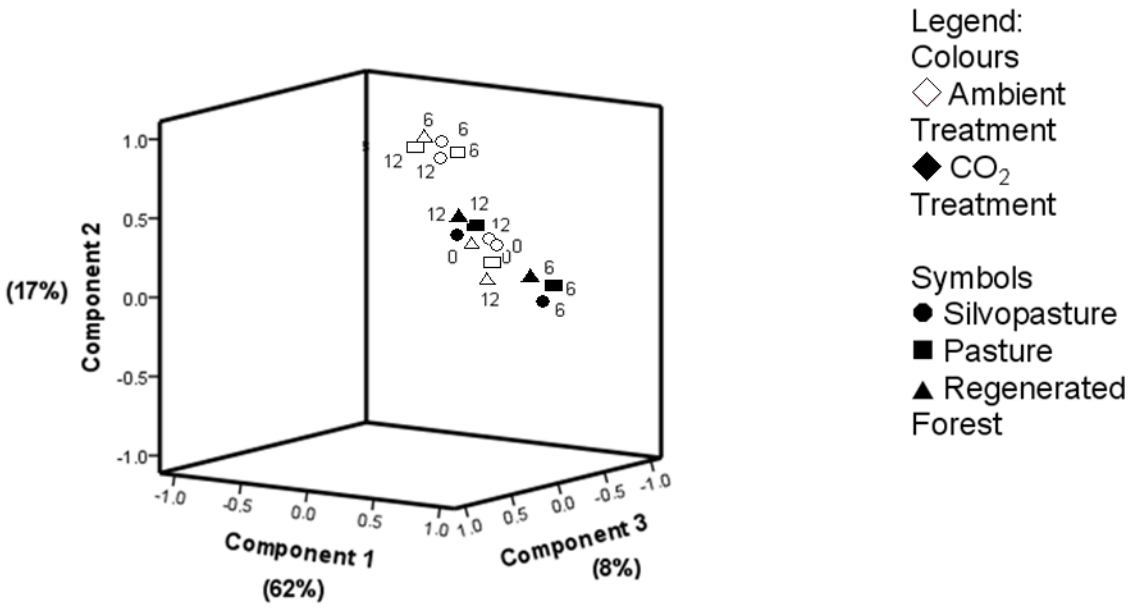

Figure 5.4 Principle components based on carbon source utilization in Biolog Ecoplates™ of conventional pasture, silvopasture and regenerated forest exposed to ambient (AMB: 360 ppm) and elevated CO_2 (CO2: 800 ppm) conditions at t=0, t=6 and t=12.

5.3.2 Soil carbon and nitrogen concentration under elevated atmospheric concentrations of CO_2

The concentration of soil C was significantly higher for all of the silvopasture treatments and times compared to the other treatments (Table 5.2). There was no significant difference in soil C concentration for any of the times and treatments of the regenerated forest site. Soil N concentration was significantly lower for all of the regenerated forest samples except at t=6 for the CO2 treatment (Table 5.2). The pasture soil at t=0 also contained significantly lower soil N concentration. The C:N ratio was significantly greater for the pasture site at t=0, the regenerated forest site at t=0, AMB treatment t=12 and the CO2 treatment t=12 (Table 5.2).

Table 5.2: Comparison of the carbon, nitrogen concentration, and C:N ratio of three soil sites; conventional pasture, silvopasture and regenerated forest for two treatments; ambient (AMB: 360 ppm), CO_2 (CO2: 800 ppm) at different times (t=0, t=6 and t=12). Standard errors are given in parenthesis.

Site	*Treatment*	*Time*	*C (g/kg)*	*N (g/kg)*	*C/N ratio*
		t=0	28.2 (0.76) [a]	1.6 (0.06) [b]	17.65[c]
	AMB	***t=6***	35.9 (0.75) [b]	2.4 (0.03) [a]	14.75 [a]
Pasture	***AMB***	***t=12***	33.2 (0.96) [a]	2.5 (0.03) [a]	13.46 [a]
	CO2	***t=6***	27.8 (0.35) [a]	2.3 (0.06) [a]	12.09 [a]
	CO2	***t=12***	31.5 (0.36) [a]	2.4 (0.06) [a]	13.13 [a]
		t=0	37.6 (0.21) [b]	2.6 (0.07) [a]	14.28 [a]
	AMB	***t=6***	37.1 (0.78) [b]	2.7 (0.10) [a]	13.74 [a]
Silvopasture	***AMB***	***t=12***	38.3 (0.95) [b]	2.8 (0.03) [a]	13.87 [a]
	CO2	***t=6***	36.9 (0.31) [b]	2.6 (0.03) [a]	14.01 [a]
	CO2	***t=12***	36.2 (0.87) [b]	2.7 (0.03) [a]	13.56 [a]
		t=0	13.0 (0.85) [a]	1.3 (0.03) [b]	10.29 [b]
	AMB	***t=6***	13.8 (0.43) [a]	1.2 (0.07) [b]	11.86 [a]
Regenerated Forest	***AMB***	***t=12***	10.9 (0.29) [a]	1.3 (0.00) [b]	8.36 [b]
	CO2	***t=6***	33.1 (0.32) [b]	2.2 (0.32) [a]	15.28 [a]
	CO2	***t=12***	11.3 (0.12) [a]	1.2 (0.00) [b]	9.44 [b]

Note: Significant differences between treatment ($p<0.05$) are indicated by differing lower case letters.

5.4 Discussion

The soil microbial diversity was comparable between site types and treatments as indicated by the richness index (Garland, 1997). The Shannon index is a reflection of microbial diversity by expressing the evenness in C substrate utilization (Garland, 1997). Every site experienced a significantly lower Shannon index level for the CO2 treatment at t=12 thereby indicating a lower diversity of the microbial community. In addition, both the pasture and regenerated forest sites experienced significantly lower Shannon indexes for the AMB treatment at t=6.

The diversity of conditions on the landscape of a silvopastoral system and regenerated forest should have allowed for microbial niche creation within the below ground environment encouraging a wider diversity of soil microorganisms (Hogberg and Read, 2006). As well, vegetation type is one of the main factors influencing microbial activity and the diversity in vegetation found between the sites should have caused significant differences in microbial community dynamics (Raich and Tufekcioglu, 2000; Hogberg and Read, 2006; Pinay et al., 2007). However, this study did not reflect that. The PCA analysis at t=6 indicated that clusters were formed based on treatments and not differences in sites, while the PCA analysis at t=12 demonstrated that the AMB regenerated forest sample was significantly different from the rest. This is supported by other studies; for example one study conducted in Costa Rica (Tornquist et al., 1999) comparing a silvopastoral site utilizing *Vochysia guatemalensis* D. Smith and *Vochysia ferruginea* Mart. tree species to conventional pasture found no significant differences in microbial activity between the two sites. Another study conducted in the Three Gorges Reservoir area (Yan et al., 2003) comparing agroforestry to native forest also found no significant difference in soil microbial activity or diversity between land uses. As well, a five year study on a grassland setting demonstrated that increasing the above ground plant species diversity on a landscape did not lead to increased diversity in microbial community (Gruter et al., 2006). In the context of this study, soil sampling in the silvopasture site only had one sub-

sample from the fodder bank and the contribution of trees in this space may have had a limited effect on soil microbial communities. Therefore there could be many contributing factors for the negligible difference in microbial community dynamics between the silvopasture and conventional pasture site. Vegetation type and biomass quality could have a greater influence on microbial diversity rather than the level of above ground plant diversity. However, a study by Lima et al. (2009) found that an agroforestry and conventional pasture site contained a greater diversity of N_2-fixing bacteria when compared to a pristine forest. Therefore, whether a change in land use increases microbial activity or diversity could be a factor of the tree or other crop species and the distribution of the vegetation within the system (Pandey and Singh, 2009).

The CO2 treatment caused an increase in AWCD for both the pasture and silvopasture sites at t=6 of the incubation. This could be a reflection of greater activity in the soil microbial community at this time but that the activity returns to levels similar to t=0 by the end of the 12 week span, thereby demonstrating no long term effects. This could be explained by the elevated CO_2 leading to elevated rates of labile C cycling and respiration (Carney et al., 2007); however, over time without the addition of more easily utilized C substrate the microbial activity would have reduced due to dieback (Taneva and Gonzalez-Meler, 2008). However; this is not reflected in the nutrient dynamics measured since no significant difference in C or N concentrations are measured and the C:N ratio is also not altered for these treatments. Other studies claim that a reduction in N accumulation will actually slow down microbial activity and rates of respiration near the surface and at further depths over time, allowing for the further build up of C stocks in the soil (Hu et al., 2001; Pendall and King, 2007). Further research supports that the direct effects of elevated concentrations of CO_2 alone, without the interaction with vegetation, have negligible effects on microbial community structure (Kanerva et al., 2008). The variability of possible influences on the dynamics of soil microbial communities, including varying vegetation types, land use, soil type, climate and

other factors can help explain the various findings from previous studies (Pendall and King, 2007; Kanerva et al., 2008).

The lower the C:N ratio of the soil the higher the rates of mineralization leading to greater quantities of bioavailable forms of nutrients (Manley et al., 2007). Although, both soil organic carbon (SOC) and total nitrogen (TN) are necessary for the productivity and overall fertility of the land, it is the balance they form that is the most beneficial for plant growth (Manley et al., 2007). High C:N ratios allow for the accumulation of C within the soil, however, without adequate quantities of nitrogen decomposition is reduced and bioavailable nutrient concentrations are diminished, this process is termed immobilization (Knops and Tilman, 2000). This study had the potential to reflect changes in microbial activity leading to changes in soil nutrient concentration based on rates of decomposition or other processes causing a loss of C and N. However, due to the chosen method of destructively sampling at t=6, soil from the same jars were not tested throughout the incubation and could account for changes in nutrient concentration including increases in C and N concentrations over time. The regenerated forest had significantly lower C:N ratios for t=0 and t=12 for both the AMB and CO2 treatments indicating that over the long run it is able to maintain a high level of bioavailable nutrients. The ability of the regenerated forest to maintain the most bioavailable nutrients is a reflection of the diversity of species found, including the presence of nitrogen fixing plant and tree species and the consistent levels of litterfall and biomass input, including the input from animal species. As a result of this high level of input combined with the high levels of decomposition commonly found in a tropical setting, soil fertility and soil nutrient equilibriums are maintained (de Gama-Rodrigues et al., 2007).

The conventional pasture site had a significantly higher C:N ratio at the beginning of the incubation reflecting the nitrogen deficient conditions which are often associated with this practice (Manley et al., 2007). The consistent significantly higher C concentrations in the silvopasture site did not lead to any overall difference in C:N ratio thereby demonstrating that it is better able to

maintain the nutritive balance then a conventional system. This is supported by many other studies (Kaur et al., 2000; Raich and Tufekcioglu, 2000; Sharma et al., 2004; Pinay et al., 2007). The ability of the silvopastoral system to be resilient to changes in nutrient concentration could be a reflection of the tree species found on the site and the resulting quality of leaf litter input (Pinay et al., 2007). As well, some studies have demonstrated that the species type can impact the quality and quantity of root turnover and the presence of C exudates from the roots thereby impacting the levels of SOM turnover (Silver and Miya, 2001; Pinay et al., 2007). These factors differ between a conventional pasture site where typically only one grass species is used as compared to a silvopastoral site where a combination of grass and trees are used. Overall, the study based on such a short time frame cannot be representative of the potential long term effects which climate change could have on agroforestry systems. However, microbial community responses to differing conditions can be rapid and this research was attempting to provide a short term perspective on the possible responses of the below ground fauna.

5.5 Conclusion

An understanding of the soil microbial community structure and nutrient concentration in differing land uses when exposed to the conditions of a changing climate could help inform future sustainable agricultural management practices and adaptation strategies. Although, soil microbial community dynamics demonstrated very little differences between sites, the soil nutrient concentration over the twelve week incubation demonstrated the potential capacity of silvopastoral systems to maintain adequate C:N ratios in order to maintain fertility. The regenerated forest had the best ratio between these nutrients and the pasture site demonstrated a lack of capacity to maintain levels of N. In response to elevated concentrations of CO_2 microbial community diversity showed little change; however in a field setting with the exposure to varying levels of biomass and other conditions this response could change. This study demonstrated that the type of land use is important

in terms of nutrient concentration and when taken outside of the field setting the microbial communities do not necessarily reflect responses to varying climate conditions in an accurate or real manner. Therefore, other methods which include long term observation in microbial and nutrient dynamics within a field setting should be investigated.

Chapter 6: Final Summary and Conclusions

6.1 Final Summary and Conclusions

Under global climate change scenerios, the role of agroforestry to maintain and improve soil fertility and crop production levels will be even more necessary. Therefore there may be a reliance on commonly used agroforestry tree species to continue to provide benefits in the form of physical barriers, nutritive biomass addition and enhancing physical soil properties (Nair, 1993; Rao et al., 1998; Gill and Burke, 1999; Jackson et al., 2000; Jobba´gy and Jackson, 2000; Ong et al., 2001; Jose, 2009; Nair, 2009). When grown under treatments reflecting changing climate conditions, both *Gliricidia sepium* (Jacq.) Walp. and *Cedrela odorata* L. tree seedlings expressed species specific responses. While a limited CO_2 fertilization effect was observed, the overall combined treatment resulted in an increased seedling growth for both species. Although this lack of expression of the CO_2 fertilization effect was not hypothesized at the beginning of the study it does emphasize that these species reacted differently than others studied. As well, the implications of the combined conditions include possible long-term changes in leaf nutrient concentration in *G. sepium* which could result in a lower capacity to renew soil fertility when applied as mulch.

When soil from three different land uses; silvopasture system, conventional pasture system and a regenerated forest were exposed to elevated concentrations of atmospheric CO_2 little change in microbial community structure was observed over a twelve week incubation. As well, microbial diversity did not vary by site indicating that land use may have varying impact on soil biota diversity. The nutrient concentration did vary by site demonstrating that the regenerated forest had the best C:N ratio followed by silvopasture and then the conventional pasture site. However, the CO_2 treatment had very little impact on the soil nutrient concentration.

While these studies were done separately they do focus on components which are very deeply interconnected in the field setting. As was discussed in Chapter 5, the microbial response and nutrient dynamics in the soil are very dependent on plant biomass input and the long term cycling of both C and N (Kaur et al., 2000; Sharma et al., 2004; Pandey and Singh, 2009). In turn, the extent of plant growth is very dependent on the availability of nutrients. When considered all together climate change could cause various impacts on the farm level. As the nutrient concentration in *G. sepium* leaves decrease when applied in the field this results in a decline in the levels of microbial activity and levels of decomposition. Therefore, a greater accumulation and storage of C in the soil would result causing less bioavailable nutrients for crop and forage production. While some caution must be taken when extrapolating these individual component results into a field setting, they do give some insight into the possible responses and implications of the changing climate on the ability of agroforestry to be a means of sustainable agriculture.

6.2 Recommendations for future research

This research attempts to fill some of the existing knowledge gaps with respect to the implications of climate change on the growth of tropical tree species especially those valued for agroforestry use. However, due to the species specific responses to the varying climate variables, further study with other tropical agroforestry species is required. When comparing previous studies there is an inconsistency in the expression of the CO_2 fertilization effect and the ability of temperature to enhance the fertilization effect. While the mechanism for the CO_2 fertilization effect is well understood the diversity of responses by the differing species indicates that a greater understanding of the influence of these variables on the specific pathways is required. As well, a better understanding is required of the response of trees

exposed to the combined conditions predicted with the changing climate throughout a lifetime, from germination to maturity. This study can comment on the sensitive seedling stage; however the response over the long-term is still not very well understood. The use of growth chambers in some early and current plant experiments allows for the control of CO_2 concentrations, temperature, moisture and all other variables associated with the growth of the plants. While these studies provide insight into the plant physiological responses, the lack of any of the realistic factors found in the field including pests, competition and disturbance prevent it from informing stand or ecosystem level responses. As well, from the perspective of agroforestry a focus should remain on the ability of each individual tree species to provide the product or service which makes them valuable even under differing climate conditions, this can include high nutrient litter, added value products such as nuts and fruit or the provision of structural components such as shade.

As individual tree responses are better understood, further forest stand and farm level studies are also required in order to incorporate other components including soil nutrient and microbial community dynamics. While many studies investigating the influence of CO_2 on soil processes and function exist there is a diversity of methods used causing a discrepancy when comparing studies. As well, studies to date have emphasized the importance of differing litter type and vegetation cover on the microbial community diversity and activity thereby influencing the various soil processes. This further indicates that on site field experiments would be the most appropriate in order to capture the conditions occurring in agroforestry systems. Ideally, if an ecosystem level or forest stand level experiment is the goal, field studies present the most optimum situation for understanding how all of the variables interact. In terms of climate change research the method representing the conditions the best that is currently available is free air CO_2 enrichment (FACE) and open top chambers (OTC). Both of these procedures involve a field situation within which CO_2 is

artificially pumped into an area to mimic predicted levels. FACE allows a whole stand to be enriched while OTC allows for one section, branch or stem of a tree or plant to be enriched while the rest of the plant encounters ambient conditions. Limitations to these studies include the fact that temperature cannot be mimicked and therefore the interactive effect between the variables cannot be assessed. In addition, within field settings many variables differ and it is extremely difficult to assess whether changes occur due to the enrichment of CO_2 or due to other variables. While all methods will always have some limitations the need for a better understanding of the impacts of climate change should drive the improvement of current available methods.

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APPENDICES

Appendix 1: Schedule of conditions in the controlled environmental chamber for all the treatments

Ambient

Time	Temperature (°C)	Relative Humidity (%)	Light (umol)	CO_2 (ppm)
0:00	22	82	0 (0)	380
5:35	22	82	1 (90)	380
5:45			2 (300)	
9:00			3 (450)	
12:00			4 (615)	
13:30	32	60	4 (615)	380
15:00			3 (450)	
17:00			2 (300)	
17:35			1 (90)	
18:00			0 (0)	
22:00	22	82	0 (0)	380
23:59	22	82	0 (0)	380

Elevated Temperature

Time	Temperature (°C)	Relative Humidity (%)	Light (umol)	CO_2 (ppm)
0:00	25	82	0 (0)	380
5:35	25	82	1 (90)	380
5:45			2 (300)	
9:00			3 (450)	
12:00			4 (615)	
13:30	34	60	4 (615)	380
15:00			3 (450)	
17:00			2 (300)	
17:35			1 (90)	
18:00			0 (0)	
22:00	25	82	0 (0)	380
23:59	25	82	0 (0)	380

CO_2

Time	Temperature (°C)	Relative Humidity (%)	Light (umol)	CO_2 (ppm)
0:00	22	82	0 (0)	800
5:35	22	82	1 (90)	800
5:45			2 (300)	
9:00			3 (450)	
12:00			4 (615)	
13:30	32	60	4 (615)	800
15:00			3 (450)	
17:00			2 (300)	
17:35			1 (90)	
18:00			0 (0)	
22:00	22	82	0 (0)	800
23:59	22	82	0 (0)	800

Combined (Temperature and CO_2)

Time	Temperature (°C)	Relative Humidity (%)	Light (umol)	CO_2 (ppm)
0:00	25	82	0 (0)	800
5:35	25	82	1 (90)	800
5:45			2 (300)	
9:00			3 (450)	
12:00			4 (615)	
13:30	34	60	4 (615)	800
15:00			3 (450)	
17:00			2 (300)	
17:35			1 (90)	
18:00			0 (0)	
22:00	25	82	0 (0)	800
23:59	25	82	0 (0)	800

Appendix 2: Tree number order within the environmental chambers for all treatments (ambient, temperature, CO_2 and combined)

Ambient

WEEK 1			Back of Chamber			
1	2	3	LIGHT SENSOR	4	5	6
7	TEMP SENS	8	9	10	11	12
		13	14	15	16	
WEEK 2						
5	14	1		12	9	4
6		15	8	2	7	10
		16	13	3	11	
Week 3						
11	10	1		2	5	8
4		16	7	9	13	14
		6	3	12	15	
Week 4						
5	2	12		15	8	9
11		10	7	14	3	6
		13	4	16	1	
Week 5						
14	7	6		8	3	12
4		13	9	5	10	16
		15	1	2	11	
Week 6						
12	5	15		9	8	10
3		7	2	13	11	16
		14	6	1	4	

Please note: numbers indicate tree number

Temperature Treatment						
Week 1						
1	2	3		4	5	6
7		8	9	10	11	12
		13	14	15	16	
Week 2						
11	9	13		14	5	2
4		3	6	15	7	1
		16	8	12	10	
Week 3						
13	3	14		8	10	7
16		2	6	11	4	12
		9	15	5	1	
Week 4						
8	4	6		7	9	12
10		11	5	3	13	1
		15	16	14	2	
Week 5						
4	6	13		5	9	3
14		2	16	8	11	1
		12	7	10	15	

Please note: numbers indicate tree number

TEMP AND CO2 TREATMENT						
Week 1						
1	2	3		4	5	6
7		8	9	10	11	12
		13	14	15	16	
Week 2						
13	11	3		4	14	5
10		2	15	6	12	9
		1	16	8	7	
Week 3						
3	14	10		1	4	15
5		16	11	8	12	2
		6	9	7	13	
Week 4						
5	9	12		4	7	16
1		13	2	14	10	8
		3	11	15	6	
Week 5						
9	16	7		8	11	2
3		1	10	6	4	14
		5	13	15	12	

Please note: numbers indicate tree number

CO2						
Week 1						
4	12	1		7	15	8
16		2	3	9	11	13
		6	10	14	5	
Week 2						
2	5	12		8	11	6
10		14	3	9	4	13
		1	16	15	7	
Week 3						
11	7	9		6	14	5
8		12	2	15	3	16
		4	13	10	1	
Week 4						
5	12	8		3	16	6
1		2	10	14	9	4
		7	15	11	13	
Week 5						
7	16	1		14	3	8
5		9	11	15	6	10
		4	13	2	12	

Please note: numbers indicate tree number

Appendix 3: Soil incubation jar order within the environmental chambers for all sites (pasture, silvopasture, regenerated forest) and treatments (ambient and CO_2)

Treatment	Jar number	Treatment	Jar number
Pasture 1	1	Silvopasture 4	10
Pasture 2	2	Silvopasture 5	11
Pasture 3	3	Silvopasture 6	12
Pasture 4	4	Regenerated Forest 1	13
Pasture 5	5	Regenerated Forest 2	14
Pasture 6	6	Regenerated Forest 3	15
Silvopasture 1	7	Regenerated Forest 4	16
Silvopasture 2	8	Regenerated Forest 5	17
Silvopasture 3	9	Regenerated Forest 6	18

Ambient Treatment

WEEK 1

Upper Level

1 2

3 4

5 6

7 8

9

Lower

10 11

12 13

14 15

16 17

18

WEEK 2

Upper		Lower	
17	16	3	10
15	18	9	7
4	14	6	5
1	2	13	12
8		11	

WEEK 3

Upper		Lower	
11	2	5	7
10	15	6	14
8	9	13	16
3	18	1	17
4		12	

WEEK 4

Upper		Lower	
1	13	11	17
10	16	12	6
5	15	18	9
14	8	3	4
7		2	

WEEK 5

Upper		Lower	
7	16	10	15
9	13	3	1
4	18	2	5
11	6	14	8
12		17	

WEEK 6

Upper		Lower	
11	14	1	7
15	5	8	17
13	4	9	10
3	18	2	6
16		12	

Week 7	Upper		Lower	
	11	5	12	17

16	4	6	10
18			

Week 8

Upper		Lower	
17	11	4	6
18	10	12	16
5			

Week 9

Upper		Lower	
5	4	18	11
16	6	10	17
12			

Week 10

Upper		Lower	
18	5	10	6
4	16	17	11
12			

Week 11

Upper		lower	
12	16	4	17
5	10	11	6
18			

Week 12

Upper		lower	
4	18	5	16
17	6	11	12
10			

CO2 treatment

Week 1

	Upper		lower	
	1	2	10	11
	3	4	12	13
	5	6	14	15
	7	8	16	17
	9		18	

Week 2

	Upper		lower	
	13	18	5	14
	2	1	8	3
	10	12	17	11
	9	6	15	7
	4		16	

Week 3

	Upper		lower	
	2	7	3	14
	10	13	8	5
	17	12	11	1
	18	6	9	15
	4		16	

Week 4

	Upper		lower	
	1	17	9	4
	11	5	13	2
	10	16	15	18
	3	7	8	14
	6		12	

Week 5

	Upper		lower	
	7	13	11	8
	6	5	9	10
	3	15	1	16
	14	12	2	18
	17		4	

Week 6

	Upper		lower	
	13	18	5	9
	3	2	1	12
	11	15	17	4
	8	6	7	10

	14		16	
Week 7	Upper		lower	
	4	5	12	16
	6	10	17	18
	11			
Week 8	Upper		lower	
	16	6	18	5
	12	17	10	4
	11			
Week 9	Upper		lower	
	10	18	4	11
	5	12	16	17
	6			
Week 10	Upper		lower	
	5	18	6	11
	17	12	10	16
	4			
Week 11	Upper		lower	
	4	12	10	5
	18	17	16	11
	6			
Week 12	Upper		lower	
	10	16	11	17
	5	18	12	4
	6			