USING STABLE ISOTOPE ANALYSIS TO MEASURE THE EFFECTS OF ENVIRONMENTAL CHANGE ON *POLYLEPIS PAUTA* IN THE ECUADORIAN PÁRAMO

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

The Effect of Environmental Change on *Polylepis pauta* in the Ecuadorian Páramo

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Tropical tree ring analysis has been a rarity in the field of dendrochronology until recent years. Trees growing along the equator experience minimal limiting growth periods due to a lack of temperature seasonality, and therefore do not produce consistent visible rings. In regions where such aseasonality produces no dormancy period, it has been found that dry seasons mimic the limiting growth periods seen elsewhere in the cold winter. Stable isotope analysis has been proven effective for identifying these yearly dry and wet periods in tropical trees. This study aims to determine if stable isotope analysis is effective for the evaluation of annual growth patterns in the northern Andes. It also aims to determine whether environmental changes, including pollution, are affecting the growth of *Polylepis pauta* in central Ecuador. In total, 68 cores were collected at four distinct *Polylepis pauta* stands. A comparative approach between isotope levels in whole wood versus cellulose is being conducted and used to determine if differences in isotope content between the two methods exist. The remaining cores were subsampled and analyzed for $\delta^{15}N$ and $\delta^{13}C$ content. Assuming the results proceed as predicted, there will be peaks in $\delta^{13}C$ corresponding to the dry months and vice versa; higher $\delta^{15}N$ values are expected to represent pollution-derived nitrogen sources. These seasonal differences will allow the reconstruction of a paleoclimate timeline and pollution history throughout the life of the tree.

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NOMENCLATURE

m.a.s.l.	Meters above sea level
sp.	Species (pl.)
DBH	Diameter at Breast Height
V-PDB	Vienna – PeeDee Belemnite
EC-JRC	European Commission, Joint Research Centre
PBL	Netherlands Environmental Assessment Agency
EDGAR	Emissions Database for Global Atmospheric Research
Kt	Kiloton

CHAPTER I

INTRODUCTION

Dendrochronology, or the study of tree rings, uses the annual rings visible on tree cores to develop chronologies which may end up stretching back for hundreds to thousands of years (McCarroll and Loader 2004). Conventional dendrochronology uses the width of both light rings (earlywood) and dark rings (latewood), which together form a yearly repetitive pattern, to develop individual tree growth histories. These analyses are then combined to reconstruct past environmental changes at the local and regional scales, which can lead to large-scale trend and comparison studies across the globe (Cook *et al*. 2004).

For many years, dendrochronologists regarded the tropics as an impossible region to study tree rings, as tropical trees do not experience dormancy periods and are thus free of latewood. In 2004, however, this mentality was broken when Poussart *et al.* (2004), published the first record of annual trace element variations in the ringless tropical tree forests of Thailand and Indonesia. They used stable isotope analysis to quantify relative changes in the abundance of carbon ($\partial^{13}C$) and oxygen ($\partial^{18}O$) isotopes, two byproducts from photosynthesis that remain stable throughout the life of the tree. Since the publication of this study, many individuals have adopted and adapted these methods for studies in Thailand, South America, and Africa. Three studies have delved further into isotope signatures, and investigated $\partial^{15}N$ signatures in tree cores from tropical species in Brazil, Thailand, Bolivia, Cameroon, and Panama (Hietz, Dünisch & Wanek 2010; Hietz et al. 2011; Sleen et al. 2015). However, these studies have used overall trends in ^{15}N enrichment (relative to ^{14}N) and do not identify small trends in ^{15}N enrichment and depletion that would indicate annual growth patterns. This study is unique because of its use of *Polylepis pauta*, a species which has never been studied in a paleoclimate reconstruction, because of its close proximity to the geographic equator, because of its use of $\partial^{15}N$ to identify growth patterns, and because of the nature of the páramo which exhibits minimal differences in precipitation amounts throughout the year.

The páramo is an Andean ecosystem characterized by high elevation (3000 – 5000 m.a.s.l.), high humidity (70 – 85%), high precipitation (3 m/yr) and low temperature (~6°C year-round) (Buytaert *et al*. 2006). It is considered a highly valued ecosystem due to the large volume of freshwater obtained from lakes formed throughout these high-altitude regions, as well as the considerable capacity for carbon storage throughout the uplands and peatlands of this ecosystem (Hribljan et al. 2017). Although very little tree growth is possible in these conditions, *Polylepis* sp. have been found to thrive in the high altitude and low temperature conditions of the páramo ecosystem. In Ecuador and throughout South America, the treeline is formed by *Polylepis* (Cierjacks *et al.* 2007). There are 18 known species within the genus, all of which grow within the high Andes. While most species can be found in Ecuador, Peru, and Bolivia, native populations have also colonized the treelines of northern Venezuela and Chile, as well as northwestern Argentina. These trees experience no dormancy period but the known limiting growth factors include low precipitation and freezing temperatures (Rehm and Feeley 2015). Seven species of *Polylepis* inhabit Ecuador and can be found forming the treelines of the páramo ecosystem. Two of these, *Polylepis pauta* and *Polylepis incana*, are the most common species that grow within the central Ecuadorian Andes. Both of these species are also found in Peru and Bolivia. *Polylepis pauta*, the larger of the two, can grow up to 12 meters and is distributed from 3400 to 4200 m.a.s.l. (**Figure 1**). The leaves of this species contain four to six pairs of leaflets and are oblong to ovate with pubescence prominent on the veins of the lower leaf surface (Romoleroux *et al*. 1996).

Polylepis pauta is considered vulnerable in Ecuador largely due to anthropogenic activities including grazing, burning, and wood extraction (Cierjacks *et al.* 2008).

Figure 1. *Polylepis pauta*. Image of a *Polylepis pauta* individual.

This study aimed to determine whether tree cores from *Polylepis pauta* can be used for dendrochronological and environmental change analysis using trace elements and, subsequently, to assess the sensitivity of *Polylepis pauta* growth patterns using trees from the dry versus humid sides of Cayambe-Coca National Park in central Ecuador. The success of this study has implications for dendrochronological research in ecosystems similar to the páramo, and for paleoclimate reconstructions done in geographical locations close to the equator. In addition, the results of this study will therefore allow further research to be done into growth patterns and limitations of *Polylepis pauta* for the benefit of its conservation as well as the conservation of the páramo.

CHAPTER II

MATERIALS & METHODS

2.1 Study sites and tree core collection

Tree cores were collected using an increment tree borer in the Cayambe-Coca National Park near Papallacta, Ecuador (**Figures 2 & 3**). In total, 68 cores were collected from trees of 8-15 inch DBH. At each of four altitudes (3611, 3852, 3941, 4033 m.a.s.l.), 16-17 tree cores were collected with at least 10 meters distance between cores to ensure two branches were not sampled from the same trunk (a common mistake when sampling *Polylepis* sp). Cores collected on the west (or dry) side of the mountain were collected from 3611 and 4033 m.a.s.l. Those collected on the east (or damp) side of the mountain were from the altitudes of 3852 and 3941 m.a.s.l. After collection, the cores were air dried for approximately one week, then stored in plastic straws until shipment from Ecuador was possible. Cores from the 3611 m.a.s.l. site location were discarded due to the presence of burn scars which were expected to interfere with subsequent $\partial^{13}C$ analysis. Nine cores from the remaining three sites were shipped from Ecuador for paleoclimate analysis. Upon arrival to the US, select cores were rewetted in order to unbend those that had dried improperly.

Figure 2. Collection Sites in Ecuador. Map indicating where collection sites are located in relation to South America.

Figure 3. Collection Sites. More detailed map indicating location of collection sites in central Ecaudor.

2.2 Core processing

Initially, one core was mounted in a wooden holder and sanded down to determine if consistent annual rings were microscopically visible on a smooth surface. Upon determination that scant rings were present, two samples were used for comparative analysis of cellulose and whole wood for $\partial^{13}C$ and $\partial^{15}N$ content of each subsample. The first core was sliced into 7 subsections, each 2.5 cm in length using a razor blade. The other core was cut into 18 subsections, each 1 cm in length. Each subsection was cut into thin slices and ground using a Retsch Mixer Mill MM400 at 25 Hz for four to six minutes until homogenized. Five additional cores (one from the 3852 m.a.s.l. site, one from the 3941 m.a.s.l. site, and three from the 4033 m.a.s.l. site) were then sliced using a razor blade into 0.5 centimeter subsamples and homogenized using the same method. After homogenization, subsamples from each core were divided in half, with one half extracted for cellulose and subsequent $\partial^{13}C$ analysis and the other analyzed for whole wood $\partial^{15}N$ content.

2.3 Cellulose extraction

Whole wood samples were extracted for cellulose content using a modified version of the Loader's lab method (Loader et al. 1996). Homogenized whole wood samples were placed into plastic chromatograph columns and saturated with distilled water. Samples were then drained of water and left to oxidize in acidified sodium chlorite bleach solution at 80°C for 50 minutes. The acidified sodium chlorite bleach solution was made up of 8.55 mg of sodium chlorite, 6 mL of acetic acid, and 600 mL of distilled water. The samples were subsequently drained of the bleach solution and soaked an additional three times using the same methodology. After the fourth soak, samples were washed once with boiling distilled water and another four times with room temperature distilled water. Samples were then soaked in 10% sodium hydroxide solution at 70°C for 45 minutes, washed with 17% sodium hydroxide solution (**Figure 4**), and finally washed another five times with room temperature distilled water. After all water was drained from the samples, they were transferred to Eppendorf tubes which were then filled with 2 mL of distilled water. Samples were homogenized using a 2mm homogenization probe on 0.5 cycles at 100% until the liquid in the samples appeared to have a translucent and slightly thick consistency. They were stored in a freezer until space became available, then freeze-dried at -50°C for 48 hours. Afterward, the dry samples were analyzed for $\partial^{13}C$.

Figure 4. Cellulose Extraction. Image of whole wood samples being drained of sodium hydroxide solution prior to being washed with distilled water.

2.4 Stable isotope analysis

Isotopic analysis of the samples was conducted in association with the Texas A&M Stable Isotope Geosciences Facility using a gas chromatograph-combustion-isotope ratio mass spectrometer coupled with an additional absorption valve to remove excess carbon signatures that would otherwise interfere with the $\partial^{15}N$ signature. This devise identifies isotopic compounds using gas chromatography prior to quantifying isotopic composition using combustion in combination with mass spectrometry. Stable isotope values are reported in parts per thousand, or per mil (‰), as a ratio of the abundance of the heavy to the light isotope; for nitrogen and carbon the ratio is $^{15}N/^{14}N$ and $^{13}C/^{12}C$, respectively. $\partial^{15}N$ and $\partial^{13}C$ in the samples are measured relative to their abundance in atmospheric conditions and described in terms of depletion or enrichment of the heavy isotope. Nitrogen isotopic abundance is measured using N_2 gas and reported in reference to atmospheric nitrogen (purified air), with a $\partial^{15}N$ value of 0‰ (Sharp 2007). Analysis of $\partial^{15}N$ was done using homogenized whole wood samples of 15 ± 1 mg each (weighed out using a microbalance) and corrected for the relative amount of sample included. Carbon isotopic abundance is typically reported in reference to the V-PDB calcite standard (which has a $\partial^{13}C$ value of 0‰) and in relation to the atmospheric $\partial^{13}C$ content (Sharp 2007). Equation 1 demonstrates how $\partial^{13}C$ is typically calculated. Analysis of $\partial^{13}C$ was done using homogenized cellulose extracted samples of 0.250 ± 0.050 mg and also corrected for the relative amount of sample included.

Equation 1

$$\partial^{13}C = \frac{(\partial C_{atm} - \partial C_{plant})}{(\partial^{13}C_{plant}/1000 + 1)} (‰)$$

CHAPTER III

ANTICIPATED RESULTS & DISCUSSION

Due to unforeseen time complications in regard to importation of the samples from Ecuador, in-lab processing of the tree core samples and subsequent isotopic analysis results were not received in time to be included and detailed in this document. This section specifies what the theoretical results will be, considering what results are already present in the literature, as well as what the implications would will be upon determination of those results. Reception of the laboratory results is imminent. A supplementary file describing and discussing the results shall be sent to the Office of Undergraduate Research Scholars in a timely manner.

3.1 Whole wood versus cellulose

Due to the high time and labor cost of cellulose extraction, as well as the need for larger sample sizes, an increasing number of studies are using whole wood to determine $\partial^{13}C$ values as opposed to extracted cellulose. These studies have found that suitable data can be obtained from whole wood; and in actuality, whole wood data may be more applicable for climate reconstructions. Better climate correlations may be found in whole wood samples because they include variation of these isotopes in the entire composition of the wood, which reinforces and often heightens the ∂ value differences observed as seasonal variation (Sleen et al. 2017). Therefore, it is likely that my results will indicate an appreciably low variation between the $\partial^{13}C$ values of whole wood and extracted cellulose. However, due to the relatively low nitrogen content of the whole wood samples, a large amount of homogenized wood was required to generate usable data for $\partial^{15}N$ and thus no data could be obtained from those samples for $\partial^{13}C$. Therefore, cellulose extraction was conducted to ensure both $\partial^{13}C$ and $\partial^{18}O$ data could be obtained at a later date and compared to the enrichment and depletion patterns observed for ^{15}N.

3.2 ∂^{15}N results

According to the EC-JRC/PBL, EDGAR version 4.3.2 (Crippa et al. 2017), N_2O emissions have increased in Ecuador from 6.15 Kt in 1970 to 16.23 Kt in 2012. This is a 62% increase in N_2O emissions in 42 years (**Figure 5**). N_2O emissions are typically the result of artificial nitrogen fertilizer applications, as well as the burning of fossil fuels. Although the consequences of these increases in nitrogen are unclear, it is reasonable to predict that elevated nitrogen deposition in the form of fertilizers impacts the cycling of nitrogen in natural ecosystems (Vervaet et al. 2002, Sleen et al. 2015). Application of fertilizers as ammonia and ammonium move through the nitrogen cycle and increase the fractionation of ^{15}N. This can lead to ^{15}N enrichment signatures seen throughout the life of the tree and, therefore, along its core (Sleen et al. 2015). Figure 2 indicates where ^{15}N enrichment and depletion occurs in the nitrogen cycle, accompanied by predicted corresponding values. The proximity of the collection sites in this study to Quito (the relatively polluted capital of Ecuador), in combination with their proximity to agricultural farms, should suggest a significant impact of these anthropogenic activities on the cycling of nitrogen in Cayambe-Coca National Park. $\partial^{15}N$ values for the *Polylepis pauta* cores collected in this study should therefore be increasing in ^{15}N enrichment from the oldest growth to the youngest growth (or from pith of the tree to its bark) (**Figure 6**).

Additionally, the deep rooting system of *Polylepis* sp. allows it to access water and nutrients during the dry seasons by utilizing these deep rooting systems and tapping into water reserves in deeper soil. During the wet seasons, these trees will not require the use of those reserves and will instead absorb water and nutrients through their shallower rooting systems (Pierret at al. 2016). This behavior can be monitored by ^{15}N enrichment in the wood of these species. Shallow soils tend to be more depleted in ^{15}N (**Figure 7**) and thus reduce the amount of ^{15}N taken up by tree roots and stored within the wood. Deep soils tend to be more enriched in ^{15}N (**Figure 7**) and therefore increase the amount of ^{15}N taken up and stored (Vervaet et al. 2002, Pierret et al. 2016). Hence, precipitation seasonality can be observed as enrichment and depletion of ^{15}N stored in the wood of a *Polylepis* sp. The results of this study should reflect this, indicating biannual changes in $\partial^{15}N$ values. Figure 3 illustrates a theoretical *Polylepis pauta* paleoclimate reconstruction using $\partial^{15}N$ to indicate seasonality.

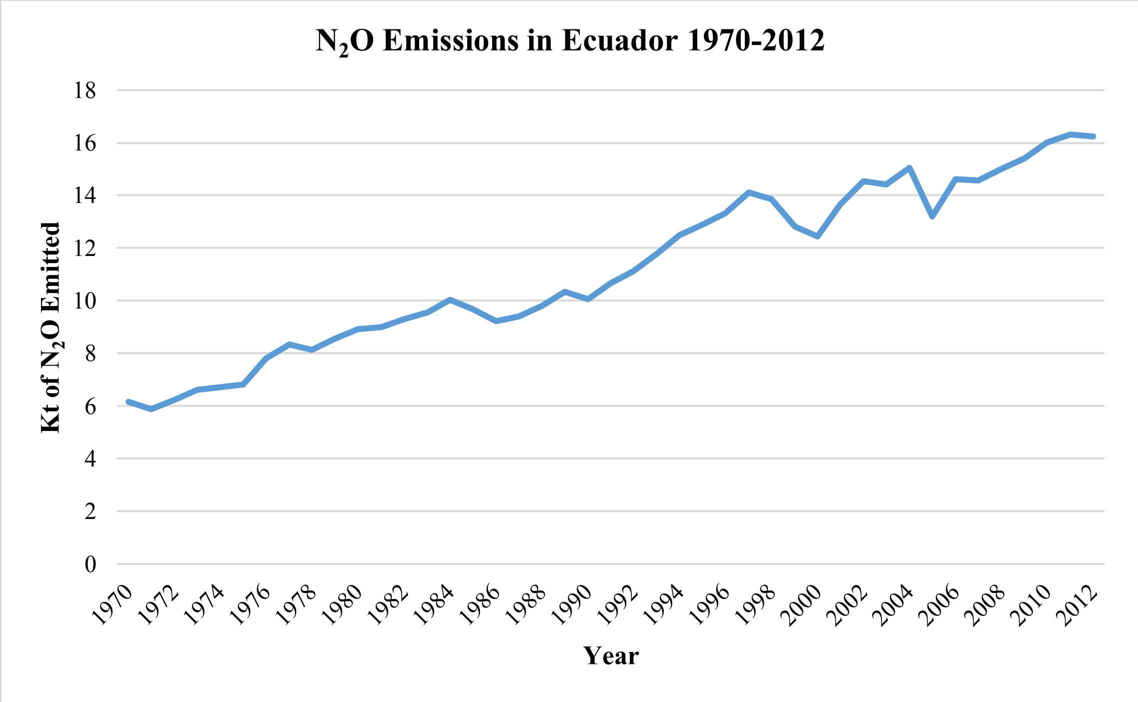

Figure 5. N_2O Emissions in Ecuador 1970-2012. N_2O emissions data from 1970 to 2012 in kilotons. This data illustrates the increasing amount of N_2O emissions in Ecuador since the 1970s. Data obtained from Crippa et al. (2017).

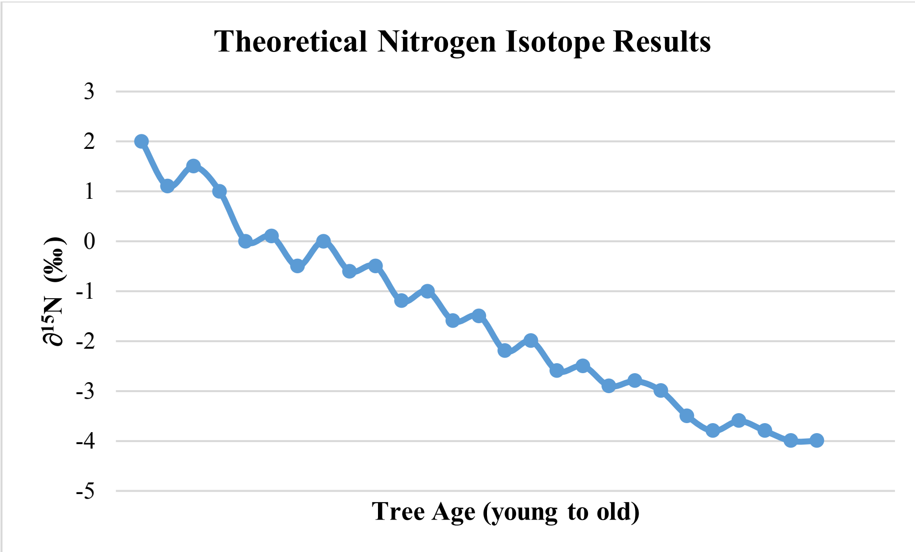

Figure 6. Theoretical Nitrogen Isotope Results. This graph represents a theoretical paleoclimate reconstruction of a *Polylepis pauta* core collected in this study. Note the progressive, long-term increase in $\partial^{15}N$ values as a result of pollution as well as the seasonal oscillations caused by the shallow vs. deep root effect.

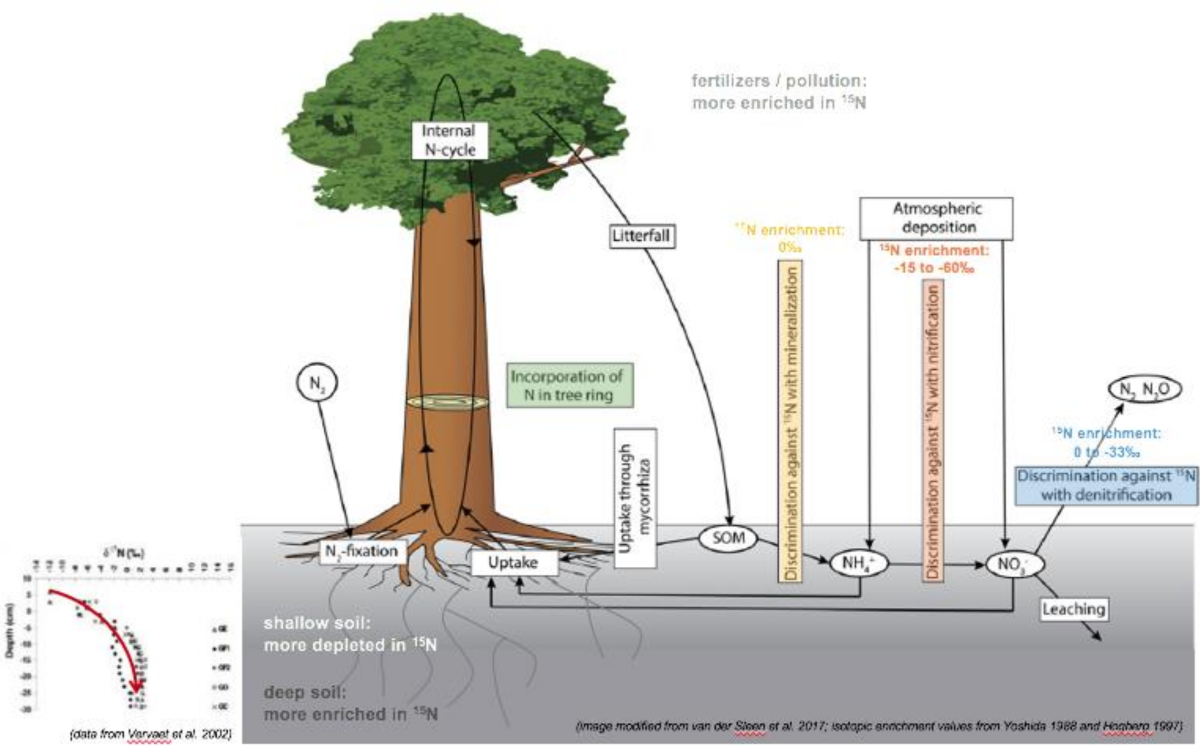

Figure 7. Nitrogen Cycle with Fractionation. Nitrogen cycle exhibiting where isotopic fractionation occurs prior to incorporation into tree ring. According to Vervaet et al. (2002)^{15}N enrichment tends to increase exponentially with soil depth. Enrichment values from Yoshida (1988).

3.2 $\partial^{13}C$ results

CO_2 emissions in Ecuador are influencing the carbon cycle in a way that is similar to what was described above for nitrogen; they have increased exponentially since the 1970s (**Figure 8**). These emissions have also increased atmospheric $\partial^{13}C$ values from a preindustrial level of -6.4‰ to a modern level of -8‰ worldwide (Sleen et al. 2017), which is referred to as the Suess Effect. This enrichment in $^{13}C_{atm}$ needs to be corrected for paleoclimate reconstructions that calculate $\partial^{13}C$ by comparing ^{13}C observed in the atmosphere to that which is observed in the plant (**Equation 1**). Even after this correction has been made, many studies are noticing a trend of ^{13}C discrimination over the last century or two (Sleen et al. 2017). This trend indicates an increase in photosynthetic activity, potentially due to warmer temperatures or increased atmospheric CO_2 concentration, because discrimination against ^{13}C occurs during CO_2 diffusion and assimilation as a function of photosynthesis (**Figure 9**). As mentioned above, the proximity of Cayambe-Coca National Park to Quito would suggest cores collected from these sites have access to CO_2 emissions from the city. Therefore, a decreasing trend in $\partial^{13}C$, or more accurately, a trend of discrimination against ^{13}C, would be visible in a paleoclimate reconstruction of a *Polylepis pauta* core from this study (**Figure 10**).

Additionally, ^{13}C has been found to be an accurate indicator of precipitation changes in temperate and tropical regions around the world (Sleen et al. 2017). It has been demonstrated for many tropical tree species that ^{13}C enrichment occurs during dry seasons due to stomatal closure (remember that ^{13}C discrimination occurs during CO_2 diffusion and assimilation through the stomata) and the opposite occurs during wet seasons (Sleen et al. 2017). It is therefore reasonable to predict that cores collected for this study of *Polylepis pauta* will exhibit this pattern, demonstrating seasonal shifts in precipitation patterns and indicating annual growth patterns (**Figure 10**). This would allow for a paleoclimate reconstruction throughout the life of the tree.

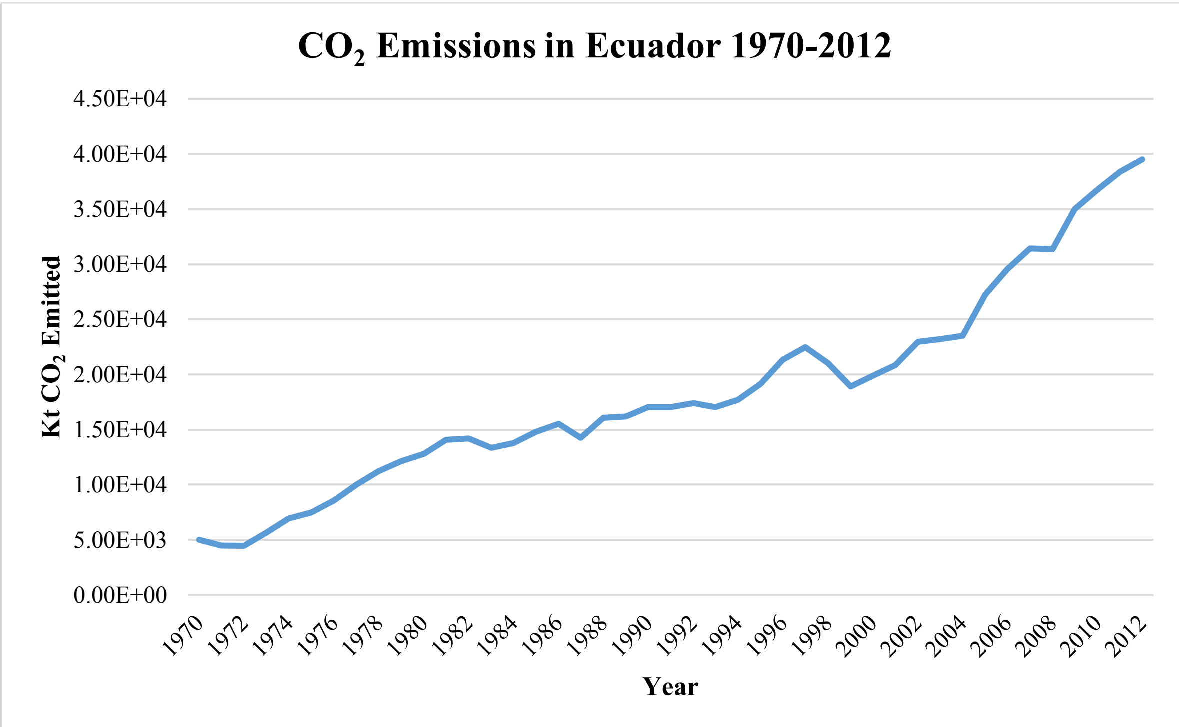

Figure 8. CO_2 Emissions in Ecuador 1970-2012. CO_2 emissions data from 1970 to 2012 in kilotons. This data illustrates the increasing trend of CO_2 emissions in Ecuador since the 1970s. Data obtained from Crippa et al. (2017).

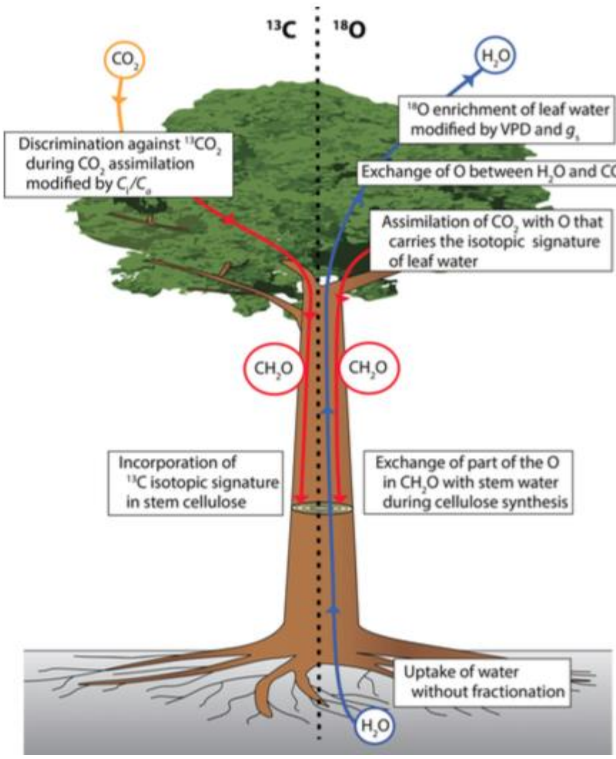

Figure 9. Carbon Cycle with Fractionation. Carbon cycle exhibiting where isotopic fractionation occurs prior to incorporation into tree ring.

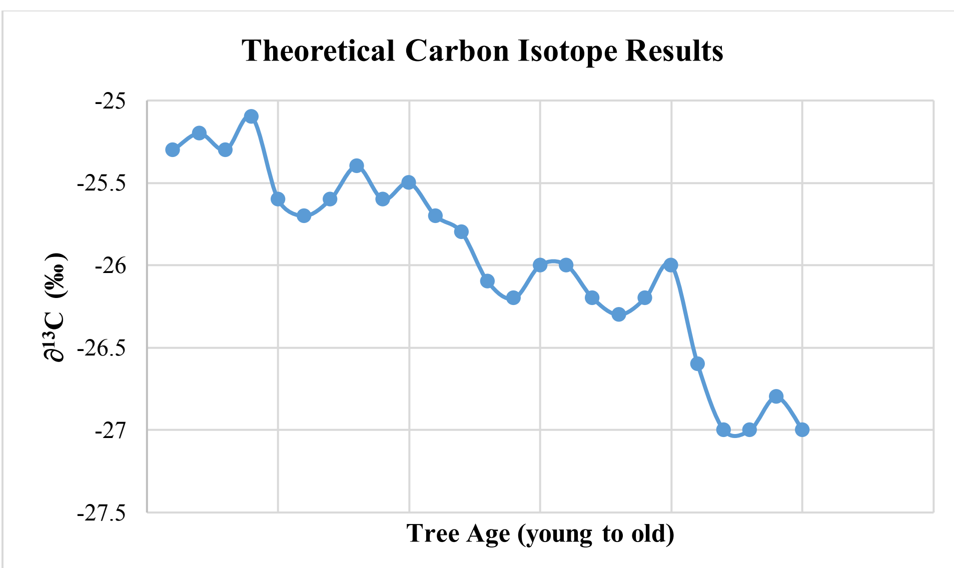

Figure 10. Theoretical Carbon Isotope Results. This graph represents a theoretical paleoclimate reconstruction of a *Polylepis pauta* core collected in this study. Note peaks in $\partial^{13}C$ values as well as the overall decreasing trend in data.

CHAPTER IV

CONCLUSION

Understanding how global environmental change is affecting the páramo has important implications for human population stability throughout Ecuador. Major cities that draw freshwater resources from páramo aboveground water sources (i.e. lakes) are vulnerable to changes in climate that reduce these water reserves. The tropics are expected to see significant changes in temperature and precipitation levels, with high elevation ecosystems projected to experience the greatest shifts in temperature and precipitation (Hribljan et al. 2017). CO_2 and N_2O emissions in Ecuador have significantly increased in the past 40 years and are likely influencing the natural cycling of nutrients (Crippa et al. 2017, Sleen et a. 2017). Understanding how these emissions are influencing the treelines in Ecuador has implications for tropical treelines worldwide. Additionally, the results from this study contribute to a better understanding of how global environmental change is influencing the natural world and what is expected to become the new normal.

Paleoclimate reconstructions of *Polylepis pauta* are valuable and contribute to a better understanding of the genus and species, what its growth patterns look like, and what is influencing those growth patterns. If the results proceed as predicted, it will be clear that nitrogen source (pollution and soil depth) as well as temperature-sensitive photosynthetic processes have a strong influence on tree growth throughout the dry and wet seasons. This has implications for the understanding of treeline behavior not only in Ecuador, but also in Bolivia and Peru.

Future studies hoping to do paleoclimate reconstructions of *Polylepis* sp. should be cautious of the extreme amount of time and labor required to obtain this data. Future core sampling using an increment borer should be done below breast height on the trunk to ensure that a core is consistently being taken from the oldest part of the tree as opposed to a branch. Sampling would be best done on a secondary forest if possible. This would allow for a general idea on the age of the trees sampled. Lab analysis should include subsampling as small as possible and isotopic analysis for $\partial^{18}O$. By measuring $\partial^{18}O$, comparisons can be made between the enrichment and depletion of $\partial^{18}O$ and those reported in this study for $\partial^{13}C$ and $\partial^{15}N$. By compiling this data with weather records for the area, a more comprehensive view of the growth patterns of this species can be recorded.

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