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Carbon Sequestration in the Cloud Forest:

A Comparative Evaluation of Aboveground Biomass Carbon Stock Potential in the Río Guajalito Reserve



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Abstract

As carbon dioxide levels in the atmosphere continue to rise at a rapid rate, it is necessary to understand how forests can both contribute to CO₂ levels but also stop them from rising. Carbon sequestration levels in tropical montane cloud forests are a relatively understudied topic. Gathering carbon stock levels is the first step necessary to start a REDD+ project. Carbon stock levels can be studied on a global, regional or local level. This study used the University of Oxford/ Global Ecosystems Monitoring Network (GEM) methodology to examine carbon sequestration levels of aboveground biomass, specifically ground litter, large branches and trees, in three different types of forest in the Río Guajalito Reserve. Ground litter and large branches were collected in order to calculate biomass from primary forest, a secondary forest from 1983 and a secondary forest from 2001. Tree biomass was calculated using an allometric equation. It was assumed that 50% of the biomass was carbon. The secondary forest from 1983 had the highest amount of carbon stock, 49.96 t C ha⁻¹, while the primary forest had the second highest, 34.4 t C ha⁻¹ and the secondary forest from 2001 had the lowest amount of carbon, 29.53 t C ha⁻¹. The secondary forest from 1983 also had the highest biomass measurement, 99.91 t ha⁻¹, signifying that of the three types of forests studied, it was the most productive, as biomass is a measure of the forest's efficiency to fix energy in all components of the ecosystem. A brief examination into REDD+ in Ecuador and how Río Guajalito would compare as a potential site was also completed.

Resumen

Mientras los niveles de dióxido de carbono en la atmósfera suben rápidos, es necesario entender cómo los bosques pueden contribuir a los niveles de CO₂ pero también bajarlos. Los niveles de la secuestración de carbono en los bosques nublados es un tópico poco estudiado. Colectar los niveles de carbono es la primera medida necesaria para empezar un proyecto de REDD+. Puede estudiar los stocks de carbono en un nivel global, regional o paisaje. Este estudio usó la metodología de University of Oxford/ Global Ecosystems Monitoring Network (GEM) para examinar los stocks de carbono de biomasa, específicamente la necromasa, ramas grandes y arboles en tres tipos de bosque diferente en la Reserva Río Guajalito. La necromasa y ramas grandes fueron colectados para que pueda calcular la biomasa del bosque primario, un bosque secundario de 1983 y un bosque secundario de 2001. La biomasa de los arboles fue calculada usando una ecuación allometric. Se asumió que 50% de la biomasa fue el carbono. El bosque secundario de 1983 tuvo el mayor cantidad de carbono, 49.96 t C ha⁻¹, mientras el bosque primario tuvo la cantidad segunda mas alta de carbono, 34.3 t C ha⁻¹ y el bosque secundario de 2001 tuvo la menor cantidad, 29.53 t C ha⁻¹. El bosque secundario de 1983 también tuvo la medida más alta de biomasa, 99.91 t ha⁻¹, la que significa que de los tres bosques, era el más productivo de todos porque la biomasa es una medida de la eficiencia del bosque para usar la energía de todos componentes del ecosistema. Una examinación breve de REDD+ en el Ecuador y cómo Río Guajalito compararía como un sitio de REDD+ fue completado al final.

Keywords: Carbon sequestration, biomass, carbon policy, REDD

Topic Codes: 614, 624, 608

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Introduction

The global climate is changing at an alarming rate. By 2100, temperature increases of 1.1-6.4 °C are projected over the 1990 level (Zhang et al., 2010). 97% of the scientific community agrees that warming trends are a result of the anthropogenic increase of greenhouse gases (GHG) (Cook et al., 2013). Between 1970 and 2004 the percent of GHG is in the atmosphere increased by 70 percent (Zhang et al., 2010). Most of these trends can be attributed to one greenhouse gas in particular: carbon dioxide (CO₂).

The concentration of CO_2 in the atmosphere is a result of a cycle between different carbon pools and CO_2 is the primary product of the oxidation of carbon from these pools



Figure 1. CO₂ levels throughout history- note sharp spikes at end (Scripps Oceanographic Institute)

(Karsenty et al., 2003). The current level of carbon dioxide in the atmosphere is 405.15 ppm and is only continuing to rise. As seen in Figure 1, in 2007 the level was at 383 ppm and has rapidly increased to arrive at the current level. Anthropogenic emissions of CO₂ originate from the burning of fossil fuels and deforestation in tropical regions (Karsenty et al., 2003). Annual CO₂ emissions grew by about 80 percent between 1970 and 2004 (Zhang et al., 2010). From 1750 to 2011. CO2 emissions from fossil fuel emissions released 375 GtC to the atmosphere, while deforestation and other land use change released 180 GtC. This has resulted in cumulative anthropogenic emissions of 555 GtC (IPCC 2013). To avoid the

predicted increases in global temperature effects we need not only lower the emissions but also find means of sequestering atmospheric CO₂ over extended periods of time (Kell, 2012).

Carbon sequestration occurs in aboveground growing biomass and in belowground soil (IPCC, 2014). The impact of carbon sequestration is site-specific and depends on factors such as local climate, tree species, and soil quality (Houghton, 2005; Pan et al., 2011). In the period of

2000-2007, global carbon absorption amounted on average to 4.1 Pg C/ year (Pan et al., 2011). This corresponds to approximately 30 percent of the emissions from fossil fuels in 2010 (IPCC, 2014). However, this absorption is often counteracted by the release of carbon in the soil from deforestation efforts (Gren & Aklilu, 2016). In forests, carbon is found in several pools including the vegetation, dead wood and litter, and soil (Fig. 2) (Karsenty et al., 2003). Over the past decade, tropical deforestation globally resulted in the release of an estimated 1.1-2.2 PgC/year (Houghton 2003; Achard *et al.* 2004; Gullison *et al.* 2007) (1 PgC = 10^{15} gC); forest degradation is thought to have resulted in similar emissions (Gaston *et al.* 1998), but data is not as abundant (but see Nepstad *et al.* 1999; Asner *et al.* 2005; Gibbs *et al.* 2007).



Figure 2. The carbon cycle in the forest

Forests are important carbon pools that are continuously exchanging with the atmosphere due to both human action and natural processes. They also act as an important and unique environmental service by removing excess carbon from the atmosphere and storing it in biomass, soils and other products (Fig. 2). However, forests also act as carbon emissions contributors when they suffer from drastic changes to climate like drought and extreme weather or when they are destroyed or degraded (Karsenty et al., 2003). While some primary forests may be carbon-neutral, forests are very rarely sources of CO2 unless they are disturbed (Luyssaert et al. 2008); therefore maintaining forests intact is critical for protecting carbon stocks while continuing carbon uptake (Mackey et al. 2014, Keith et al. 2015). A lot of interest exists in monitoring carbon in tropical forests because it is important to understand the role that these forests play in the carbon cycle at a global level and the possible implications of climate change on them.

Tropical forests in particular play an important role in the carbon cycle due to the large amount of carbon stock they store (Dixon et al., 1994). Approximately 31 percent of the carbon is stored in the biomass and 69 percent in the soil in all types of forests. In tropical forests, approximately 50 percent of the carbon is stored in the biomass and 50 percent in the soil (IPCC, 2000). Tropical forests are major sinks for atmospheric carbon, accounting for about 37% of the terrestrial carbon sequestered in aboveground biomass and soil (Martin et al., 2015). It is estimated that carbon emissions from tropical deforestation represent approximately 20% of the

total global greenhouse gas emissions generated from human activities (Denman et al., 2007). Despite the benefits, tropical forests are undergoing widespread loss, mainly as a result of agricultural expansion (Gibbs et al., 2010). These losses have led to increased carbon emissions, species extinctions and structural alteration to tropical forests worldwide (Gardner et al., 2009; Foley et al., 2007). Recent global estimates suggest that if tropical deforestation were stopped entirely, if mature forests remain undisturbed, and if new forests were allowed to continue regrowing on deforested land, 24 to 35% of all carbon emissions from fossil fuels and industrial production from 2000 to 2010 could be mitigated.

Financial mechanisms such as REDD+ (reducing emissions from deforestation and forest degradation) have been proposed as ways to encourage the conservation of tropical forests. Crucial steps in the implementation of REDD+ are to estimate national-level carbon emissions from deforestation and forest degradation and to collect data on local biomass and carbon stocks (Stas, 2014). Inventories of the carbon cycle in tropical forests are important for a variety of studies. For example, the first step in developing a REDD project is the inventory of carbon stock of the area (Honorio & Baker, 2010). Also, when one needs to understand the possible effects of climate change on carbon stock in tropical forests, one must measure different carbon flows over a period of time. The impact of carbon sequestration is site-specific and depends on factors such as soil quality, tree species, and local climate (Houghton, 2005; Pan et al., 2011). Ecuador has been a participant in the UN-REDD program since Oct 2009 when it was formally accepted as an observer country.

Ecuador has suffered from drastic deforestation. Ecuador is considered to be a megadiverse country, containing between 5% and 10% of the biodiversity of the whole world (Mittermeier et al. 1997). While Ecuador has contributed only 0.001% of global emissions responsible for climate change, it is a country highly vulnerable to the impacts of climate change because of its geographical, social and economic situation Between 1990 and 2014, Ecuador lost about 2.2 million hectares of natural forest. In 1990 the total forest area was equal to 14,587.771 hectares and in 2014 the total area 12, 753.387 hectares (Ministerio del Ambiente de Ecuador, 2016). Recent data from the Ministry of Environment, however, indicate that the national deforestation rate is significantly lower than the earlier figure and is closer to 61,765 ha per year (equivalent to 0.6 percent per year) (MAE, 2011). Over the last few decades, the Andean highlands of Ecuador have been characterized by intense afforestation efforts, in order to reduce erosion, increase the economic return of less viable agricultural areas and, more recently, to sequestrate atmospheric carbon (Buytaert et al., 2007). Ecuador is a prime location for carbon sequestration projects because 51% of the country is covered in native forest (MAE, 2016).

Objectives

In order to implement monitoring systems of carbon for projects that look for financial benefits to increase carbon stock (e.g. agroforestry project, plantations) or to reduce the amount of CO_2 emissions (e.g. REDD+) carbon stock must be studied (Honorio & Baker, 2010). In this study, the amount of carbon stock in both primary and secondary forest in the Ecuadorian cloud forest ecosystem was examined. The study set out to answer the question of whether primary forest would have the highest levels of carbon stock, measured in t C ha⁻¹. It also examined tree diversity in different types of forest and hypothesized that tree diversity would be greater in primary forest than in both the secondary forest from 1983 and the secondary forest from 2001. After data analysis, a brief analysis on how REDD+ is used in Ecuador and how it may apply to

a site like Río Guajalito.

Materials and Methods

Materials

- 50 meter long measuring tape
- DBH tape
- Laser range height finder
- Plastic string
- Plastic markers
- Multifunctional hanging scale
- 2 2-meter long poles
- Large plastic sac with rope attached
- Graduated cylinder
- Pocketknife
- Machete

Study Area

This study took place during a three-week period from mid-November to mid-December in the Río Guajalito reserve; a tropical montane forest located 48 kilometers west of Quito (78°49'W, 0°14'S, Prov. Pichincha, 3 km NW from km 59 on the old road from Quito to Santo Domingo, 1800-2000 m a.s.l.). (Fig. 3). The Río Guajalito reserve includes 795 hectares of protected forest, the majority primary forest. The reserve has multiple owners who own various amounts of the reserve. The area has been under protection as a private reserve since 1984 (Nieder & Barthlott, 2001). However, there are indications of former and current human influence within parts of the reserve such as logging, a PetroEcuador gas pipeline and cattle farming. Overall precipitation reaches up to 3,288 mm per year, with a mean of 2,738 mm (calculate from Instituto Nacional de Meteorologia e Hidrologia, Quito). Soils are typically Andosols (esp. Dystranteps) on volcanic material (Nieder & Barthlott, 2001).

Three randomly chosen sites were selected in different types of forest: one in a secondary forest from 1983, one in a primary forest and one in a secondary forest from 2001. Prior to 1983, the secondary forest was used as grassland for cattle grazing; this has led to impacts such as erosion on the ecosystem. Prior to 2001, the other secondary forest on the reserve was cut down and has been regenerating since; therefore there was no erosion.



Figure 3. Location map of the Río Guajalito Reserve (Nieder & Barthlott, 2001).

Study Design

The University of Oxford/ Global Ecosystems Monitoring Network (GEM) methodology was used during this study, a methodology that is used by sixty countries worldwide (Honorio & Baker, 2010). In each site, a quadrat of 10 m x 10 m was established. Within the quadrat, 25 2 m x 2 m plots were established using markers along the outside of the quadrat. Within each 2 m x 2 m plot, 16 50 cm x 50 cm sub-plots were established. In the 10 m x 10 m plot, 40 sub-quadrats of 50 cm x 50 cm were randomly chosen using a numbered system (Fig. 4). All the above ground biomass excluding trees and large roots was removed by hand (referred to as ground litter throughout the study) (Fig. 5). Large branches/ logs were separated from all other biomass and collected and analyzed separately. A machete was used to remove particularly large branches/ logs from the sub-quadrats. Once the A horizon of the soil was reached, biomass collection stopped.



Figure 4. Diagram of 10 m x 10 m plot with labeled subplots.



Figure 5. A 50 cm x 50 cm sub-plot before and after biomass collection

Ground litter biomass was put into a large plastic sac and weighed on the mountain using a multifunctional hanging scale with a maximum weight capacity of 25 kilograms. Once weighed, the biomass was placed in a large pile near the plot. After the biomass was collected from all 40 sub-quadrats and placed in the pile, the pile was mixed well and a representative 1-kilogram sample was taken back to the Rio Guajalito reserve station to be dried and weighed. It was dried in a herbarium plant dryer at 70 degrees Celsius for 48 hours. Once completely dried, the biomass was weighed again. According to Karsenty et al., Chave et al. and many other authors, the amount of carbon in the biomass varies from between 35 to 65 percent of the dry weight (50% is often taken as a default value) (Karsenty et al., 2003). In this case, it was assumed that 50% of the dry weight represented the carbon stock. Using the wet:dry ratio, the amount of carbon stock was calculated and converted to Mg C ha⁻¹, as carbon stock is measured in terms of weight per unit of area.

For the large branches/ logs, samples from each piece were broken up with a machete and brought back to the reserve station. A representative 1-kilogram sample was taken and dried at 70 degrees Celsius for 48 hours. Just like the rest of the biomass the dry weight was taken and was converted to Mg C ha⁻¹.

A less destructive approach was taken for measuring tree biomass. If more than half of a tree appeared in a sub-quadrat, the biomass for each tree was calculated using an allometric equation that was compiled using data from destructive harvesting of 2,410 trees from 27 study sites across the tropics (Chave et al., 2005). The equation is as follows:

AGB=0.0776*(I*(DBH2)*H)0.94(1)

Where AGB is aboveground biomass, I is density of the wood, DBH is diameter at breast height (cm) and H is tree height (m).

In order to calculate density (I), samples of each tree were collected using 2-4 meter long metal poles with a claw attached at the end. Tree samples were scraped of their bark using a

pocketknife and cut into pieces to fit into a graduated cylinder. The samples were first weighed, and then the volume was calculated using the graduated cylinder to find the density. To find DBH, a DBH tape was used in the field. To find height, a laser-range finder was used or height was estimated. Trees were identified to genus or species using samples collected by the 2-meter long poles with a claw attached at the end.

Once all the biomass measurements were calculated for ground litter, large branches, and trees, data was analyzed using bar graphs and pie charts. Shannon-Weiner index was used in order to find compare tree diversity and evenness between the three types of forest.

Results

The data shows that the secondary forest from 1983 sequesters the most carbon, 49.96 t C ha^{-1} (Fig. 6). The primary forest had the second highest amount of carbon stock; 34.4 t C ha^{-1} and the secondary forest from 2001 had the lowest amount of carbon stock, 29.53 t C ha^{-1} . As shown in Figure 6, the secondary forest from 1983 had the highest carbon stock measurements for both trees and ground litter, 18.99 t C ha^{-1} and 24.06 t C ha^{-1} , respectively. The primary forest had the highest carbon stock for branches/ logs, 10.55 t C ha^{-1} . In the secondary forest from 2001, branches sequestered 2.43 t C ha^{-1} and ground litter sequestered 11.72 t C ha^{-1} .



Total Carbon Content



Total biomass measurements for each forest are shown in Figure 7. The secondary forest from 1983 had the highest total biomass weight, 99.9 t ha¹. The primary forest had the second highest biomass measurement, 68.81 t ha⁻¹. The secondary forest from 2001 had the lowest total biomass weight, 59.05 t ha⁻¹.



Figure 7. Total biomass measurements in t ha⁻¹ (ground litter, branches and trees)

In Figure 8, biomass measurements of ground litter, branches and trees are shown on a clustered bar graph. As the graph shows, the secondary forest from 1983 had highest weight for ground litter, 48.11 t C ha⁻¹ and trees, 37.98 t ha⁻¹ and the primary forest had the highest weight for branches, 21.1 t C ha⁻¹. The secondary forest from 2001 had the lowest biomass weights for both ground litter and branches, 23.25 t C ha⁻¹ and 4.85 t C ha⁻¹, respectively. The primary forest had the second highest weight of ground litter biomass, 35.5 t C ha⁻¹ and the secondary forest from 1983 had the second highest weight for branches, 13.82 t C ha⁻¹. The primary forest had the lowest measurement for trees, 15.16 t ha⁻¹ and the secondary forest from 2001 had the second highest measurement for trees, 30.95 t ha⁻¹.



Figure 8. Clustered bar graph of biomass measurements for ground litter, branches and trees

Wet weights of biomass were also analyzed to see how they compared to the biomass measurements and dried weights. The primary forest had the highest wet weights for ground litter and branches, 130.18 kg and 95.73 kg respectively. The secondary forest from 2001 had the second highest measurements for both ground litter, 116.23 kg and branches, 33.95 kg. The secondary forest from 1983 had the lowest wet weights for ground litter and branches, 90.78 kg and 24.68 kg respectively.



Figure 9. Total wet weights of ground litter and branches across the three plots

In Figure 10, the dried weights of the representative 1-kilogram sample were analyzed using a clustered bar graph. The secondary forest from 1983 had the highest dried weights for ground litter and branches, 530 g and 560 g respectively. This signifies that for example, the ground litter in the secondary forest from 1983 loses 470 g of water in each kilogram. Primary forest had the second highest dried weights for both ground litter (250 g) and branches (270 g). Finally, the secondary forest from 2001 had the lowest dried weights for ground litter, 200g and branches, 225 g. It should also be noted that the branches dry weight was higher than the ground litter dry weight across all three plots.



Dried Weights

Figure 10. Dried weights in grams after representative 1 kilogram sample was left in the plant dryer for 48 hours at 70°C

The numbers of trees in each plot are represented in Figure 11. Not many trees were recorded due to the methodology of only counting the trees that appeared in the 50 cm x 50 cm sub-plots. The secondary forest from 1983 had the highest number of trees, 8 followed by the primary forest with 7 trees and finally the secondary forest from 2001 with 6 trees, the lowest amount.



Figure 11. Number of trees in each plot

For tree abundance in the secondary forest from 1983, there were 2 Clusia pseudomangles, 2 dead trees that were unable to be identified, 1 palicourea demissa and 2 Miconia. For tree abundance in the primary forest, there was 1 Psychotria, 1 Styrax cf. cordatus, 1 Nectandra reticulate, 1 Eschweilera caudiculata, 1 Palicourea demissa and 2 Miconia. In the secondary forest from 2001, there was 1 Miconia, 1 dead tree that was unable to be identified, 2 Cyathea, 1 Exarata chocoensis and 1 Weinmannia macrophylla (Fig. 12).

The most common tree found in the studied plots was *Miconia* (Fig. 12). A *Miconia* was found in every plot: 3 in the secondary forest from 1983, 2 in the primary forest and 1 in the secondary forest from 2001. The only other trees that appeared in multiple plots were *Palicourea demissa* and dead trees that were unable to identified. Dead trees were unable to be identified because they had lost all of their leaves.

The primary forest had the highest number of tree species, 6. The secondary forest from 2001 had the second highest number of tree species at 5 species. The secondary forest from 1983 had the lowest number of tree species with 4 species.



Tree Distribution

Figure 12. Tree species distribution across all plots

Figure 13 shows the percentages of carbon stock from each tree for each type of forest. In the secondary forest from 2001 *Weinmannia macrophylla* contributed the most to the carbon stock, contributing 48% of the carbon stock of all the trees measured in the secondary forest from 2001. The tree that contributed the second highest amount of carbon stock was *Cyathea* at 25% of the total. *Exarata chocoensis* was 19% of the total, *Miconia* was 5% of the total and dead trees that were unable to identify contributed the least at 3% of the total. In the primary forest *Miconia* contributed the most to the total carbon stock, at 52% of the total. *Styrax cf. cordatus* was the second highest percentage at 21%. *Eschwilera caudiculata* was 12% of the total, and *Nectandra reticulate*, *Palicourea demissa* and *Psychotria* each contributed 5% to the total tree carbon stock. The second highest percentage was *Clusia pseudomangle* at 42%. Next were dead trees that were unable to be identified at 14% and last was *Palicourea* at 1% of the total tree carbon stock.





Shannon-Wiener indexes were calculated for each plot as shown below in Table 1. The secondary forest from 1983 had the highest H-max number, 2.08 and the highest evenness number, 0.64. The primary forest had the second highest H-max number, 1.95 and the lowest evenness number, 0.14. The secondary forest from 2001 had the lowest H-max number, 1.79 and the second highest evenness number, 0.17.

Table 1. Shannor	Diversity	of tree	species	across all	three	plots
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Plot	H Max (Shannon Diversity)	Evenness
Secondary Forest- 1983	2.08	0.64
Primary Forest	1.95	0.14
Secondary Forest- 2001	1.79	0.17

Figure 14 shows qualitative differences between each forest. Fig. 14a is a photo of the secondary forest from 1983. The leaves here are dry and there isn't as much plant life growing in/ around the sub-plot. Fig. 14b is from the primary forest. The area is much damper and there are more low-growing plants surrounding the area. Fig.14c is from the secondary forest from 2001. This was the last plot completed and as the picture shows, the ground was very wet before collecting the biomass from the sub-plot.



Figure 14. Pictures of 50 cm x 50 cm sub-quadrats at each site. **a.** secondary forest from 1983. **b.** primary forest **c.** secondary forest from 2001

Discussion

Carbon

The main conclusion of the study is that the forest with the highest amount of carbon stock was the older secondary forest, from 1983, which had a total carbon stock content of 49.96 t C ha⁻¹ (Fig. 6). The primary forest had the second highest amount of carbon and the secondary forest from 2001 had the lowest amount of carbon. This means that the hypothesis of the primary forest sequestering the most carbon was not proven true, as the secondary forest from 1983 sequestered the most carbon. It was hypothesized that primary forest would have the highest amount of carbon stock because it is the oldest type of forest on the reserve and therefore would have more biomass on the forest floor, larger fallen branches and larger trees. However, not all of this was true for the randomly chosen plot in the primary forest. This could have been because the primary forest is an established forest, therefore not as much biomass is moving through it compared to the two secondary forests. Also, it is possible that because the study was focused on the understory and not the more diverse canopy area

The extent of carbon storage and sequestration in tropical montane forests is still relatively poorly understood (Spracklen & Righelato, 2016) and even more poorly understood in secondary forests. Estimates of the carbon sequestration potential of naturally re-growing forests

have been hampered by the lack of spatially explicit information on the extent and age distribution of secondary forests and on the effects of climate and other environmental factors on local rates of biomass recovery (Chazdon et al., 2016). According to Krankina & Harmon, Bryan et al. Keith et al., and Carlson et al., primary forests store 30–70% more carbon than logged and degraded forests. The main reason for higher carbon stocks in primary forests is that most living biomass carbon is found in large, old trees and in undisturbed soil stocks (Stephenson, 2014). According to this observation by Stephenson et al., it would make sense why the primary forest plot didn't have higher carbon stocks than the secondary forest from 1983. There were no large trees measured in the primary forest plot; the tree with the largest DBH and height was the *Styrax cf. cordatus*, with a DBH of 27.9 cm and height of 7.41 whereas in the secondary forest from 1983 there was a *Clusia pesudomangle* with a DBH of 33.4 and a height of 14. This data could have skewed tree carbon stock measurements. Also, soil was not measured in this study, but if it had been primary forest may be more likely to have the higher biomass measurements due to the soils being less disturbed than in a secondary forest.

Biomass

The productivity of each site was indicated by biomass. Biomass is a measure of the forest's efficiency to fix energy in all components of the ecosystem (Rochow, 1974). The secondary forest from 1983 had the highest biomass calculations, 99.9 t ha⁻¹. The secondary forest 2001 had the lowest biomass measurement, 59.05 t ha⁻¹, which is logical considering it is the youngest of the three forests.

Figure 14 shows qualitative differences between the three types of forest. In the primary forest and secondary forest from 2001 there was an overall thinner layer of biomass on the ground but more plants growing. It's possible that this is why the total wet weights of biomass were higher in the primary and secondary forest from 2001 than the secondary forest from 1983 (Fig. 9). These two forests were also much wetter than the secondary forest from 2001. This could be another reason why in Figure 9, the primary and secondary forest from 2001 had such higher total wet weights than the secondary forest from 2001; the primary forest had a total wet weight of ground litter and branches of 208.34 kg and the secondary forest from 1983 had a total wet biomass weight of just 115.46. It is unclear whether this was due to the fact that it didn't rain much the first week of data collection when the secondary forest from 1983 was completed or whether it's because the secondary forest from 1983 was closer to a cattle grazing field that received a lot of sunlight and was therefore drier than the other two types of forest.

It's also important to note how many more branches/ logs were found in the primary forest. The total weight for branches in the primary forest was 78.16 kg, more than three times heaver than the branches found in the two types of secondary forest. This could be due to the fact that the fallen branches and logs that came from the primary forest came from very large trees who had established themselves in the plot many years ago. Trees in the secondary forests wouldn't have had a chance to grow as tall as some of the trees in primary forest.

Although the secondary forest 1983 plot had the lowest weight of total wet ground litter and branches, 115.46 kg (Fig. 9), it had the highest dried weights for ground litter and branches, 530 g and 560 g respectively (Fig. 10), after a representative 1-kilogram sample was dried in the plant dryer. It can be speculated that this happened because there was very little water in the biomass due to essentially no rainfall happening the week data collection happened in that plot. With no water being absorbed by the biomass, it most likely made the biomass lighter. The secondary forest from 1983 also had the highest measurement for tree biomass, 37.98 t ha⁻¹, while the primary forest had the lowest measurement for tree biomass, 15.16 t ha⁻¹. The primary forest had the highest wet weight of ground litter; 130.18 kg and the secondary forest from 1983 had the lowest wet weight for ground litter, 90.78 kg. Therefore, biomass measurements (t ha⁻¹) do not necessarily correlate with wet weight (kg) calculations. According to the FAO, carbon content of litter should usually be determined from field samples since some of the material may be extremely decomposed and the carbon content may differ from that of less decomposed material. Application of a ratio approach will often underestimate carbon content of the litter layer due to the escape of carbonic gases during the process of decomposition (FAO, 2005).

There is much debate over the usefulness of secondary forests as carbon sinks and places of high biodiversity. According to a study done by Turner (1997), the secondary forest, despite a century or so for colonization by species and the presence of contiguous primary forest, was significantly less diverse than primary forest. Turner concludes that secondary forest cannot be assumed to accrete biodiversity rapidly in the tropics, and may not be of direct value in conservation (Turner, 1997). But Chazdon et al., argue that coupled with avoided deforestation and sustainable forest management, natural regeneration of second-growth forests provides a low-cost mechanism that yields a high carbon sequestration potential with multiple benefits for biodiversity and ecosystem services. Locatelli et al. have also determined that secondary tropical forests are important carbon sinks and restoration of these forests can be an important climate change mitigation option (Locatelli et al., 2015). Rates of biomass accumulation in secondary forests are typically faster in regions with high surrounding forest cover (Bonner et al., 2013), so perhaps this was why the secondary forest from 1983 had a higher biomass measurement than the primary forest (Fig. 6).

Table 2 provides a brief synopsis of aboveground biomass measurements (t ha⁻¹) of different types of forests around the world compared with the measurements from this study. As shown in the table, the amount of biomass in each forest varies greatly. Different allometric equations were used in most of the studies. This data confirms that each type of forest has the ability to sequester extremely different amounts of carbon. Although not every study compares secondary and primary forest, there are still great differences between each the different study sites. For example, Taita Hills, Kenya had a total aboveground biomass measurement of 767 t ha⁻¹, while a primary wet montane forest in Costa Rica (an ecosystem fairly similar to Río Guajalito) had a measurement of 179 t ha⁻¹.

Ecosystem	Biomass (aboveground biomass)
Primary Forest Río Guajalito	$68.81 t ha^{-1}$
Secondary Forest 1983 Río Guajalito	99.9 t ha^{-1}
Secondary Forest 2001 Río Guajalito	$59.05 t ha^{-1}$
Mangroves in Latin America	78 t ha ⁻¹ (Estrada & Soares, 2017).
Wet Montane Forest, Costa Rica: secondary	2.6 t ha ⁻¹ (Tanner et al., 2016)
forest, six years old	
Wet Montane Forest, Costa Rica: primary	179 t ha ⁻¹ (Tanner et al., 2016)

Table 2. Comparison of different forest ecosystem aboveground carbon stock measurements

forest	
Wet Montane Forest, Costa Rica, secondary	21.4 t ha ⁻¹ (Tanner et al., 2016)
forest, 13 years old	
Dry Evergreen Forest	198.20 t ha ⁻¹ (Mani and Parthasarathy, 2007)
Mixed Deciduous Forest	96.28 t ha ⁻¹ (Terakunpisut et al., 2007)
Upper montane wet forest, South-Ecuador	149 t ha ⁻¹ (Hofstede & Aguiree, 1999)
Pifo Polylepis forest, Pichincha province,	366 t ha ⁻¹ (Feshe et al., 2002)
Ecuador	
Metrosideros stands, Hawaii	81-123 t ha ⁻¹ (Raich et al., 1997)
Montane tropical forest, SE Peru	94-205.6 t ha ⁻¹ (Girardin et al., 2010)
Tropical montane forest, southern Ecuador	104-158 t ha ⁻¹ (Spracklen et al., 2016)
South Bhadra, Karnataka, southern India	649 t ha ⁻¹ (Rai & Proctor, 1986)
Taita Hills, Kenya	767 t ha ⁻¹ (Omoro et al., 2013)
Podocarpus National Park, Ecuador	88.1-256 t ha ⁻¹ (Moser et al., 2011)
	(Leuschner et al., 2007)
Montane moist forest, Rio Grande, Venezuela	294 t ha ⁻¹ (Delaney et al., 1997)

Trees

Tree species distribution among the forests also provided interesting results. Because this study only looked at three 10 m x 10 m plots with only 10% of those plots being surveyed, tree data is most likely skewed. Although the 50 cm x 50 cm sub-plots were chosen randomly, this could have meant that in one type of forest more trees appeared in the sub-plots than in another. Figure 11 displays the number of trees surveyed in each plot, with the max number of trees just 8 in the secondary forest from 1983. Nevertheless, tree data was still collected, as tree biomass is an important determinant in carbon stock. As mentioned above, the FAO points out that the carbon content in ground litter is often underestimated due to the escape of carbonic gases during the process of decomposition. Therefore it is important to note the important role that trees play in forest carbon storage by acting as major sinks of atmospheric carbon (Karthick et al., 2014).

Miconia was the only tree that was found in all three plots (Fig. 12). The highest number of *Miconia*, 3, was found in the secondary forest from 1983. The primary forest had the highest number of different species in the plot- a total of 6 different tree species. This is not a surprise, as it is generally known that primary forests host a greater number of tree species than secondary forests. However, this does not necessarily make the primary forest more diverse than the secondary forest plots. When the Shannon Diversity was calculated for all three plots, the secondary forest from 1983 had the highest H-max, 2.08 (Table 1). The primary forest H-max was 1.95, so not a drastic difference between the two but is important to realize that the primary forest was not the most diverse. If more plots had been studied, it could be helpful to use other diversity indices and compare them across more plots. There were also a considerable amount of dead trees that could not be identified in the secondary forest from 2001 and the secondary forest from 1983. No dead trees were found in the primary plot. This is probably due to the fact that there are less pioneer species in primary forests that have very short life spans, compared to primary species, which can have life spans of 50-100 years (Startin, 2015).

As mentioned, the plot with the highest number of trees (8) was the secondary forest from 1983 (Fig. 10). Therefore, it seems as if the highest H-max, highest evenness and the highest

biomass also had to do with the amount of trees in the plot. However, this same pattern does not work the same way for the primary and secondary forest from 2001. The primary forest had the second highest number of trees, 7, the second highest H-Max number, 1.95 but the lowest evenness number, 0.14 and the second lowest biomass among the three plots. The secondary forest from 2001 had the lowest number of trees, most likely because it has had the least amount of time to establish itself compared to the other two forests. According to Wood (2011), species diversity is expected to increase over time as ecological succession proceeds. This is proved true when comparing the secondary forest from 2001 to the other two forests, but not when comparing the secondary forest from 1983 to the primary forest. According to most other research, primary forest should have the highest diversity and also the highest biomass. Perhaps this was not the case because there was only one plot that could be completed in each type of forest. This did not allow for as much randomization as other studies that use multiple plots in primary and secondary forest.

Percentage of carbon stock from each species of tree in each plot was also analyzed in order to see which type of tree species had the highest amounts of carbon stock (Fig.13). Figure 13a. represented carbon stock percentages for the secondary forest from 2001. *Weinmannia macrophylla* had the highest percentage of the secondary forest from 2001, almost half of the total at 48%. *Miconia* and dead trees were the smallest contributors to the total biomass of trees in the secondary forest from 2001, with 5% and 3% of the total respectively. However, in the primary forest and the secondary forest from 1983 *Miconia* made up the majority of total biomasses, 52% and 43% respectively. This is most likely due to the fact that the *Miconia* found in the secondary forest from 1983 and the primary forest. *Clusia pseudomangle* was also a large contribution to total biomass for the secondary forest from 1983, with 42% of the total tree biomass. The dead trees contributed a higher percentage in the secondary forest from 1983 than in the secondary forest from 2001. This is most likely due to the fact that there were 2 dead trees in the secondary forest from 1983 and only 1 dead tree in the secondary forest from 2001 (Fig. 12).

Policy Implications

Recognition of the need to stabilize the carbon content in the atmosphere has been emphasized in a number of international and national agreements and policies, such as the Kyoto Protocol, the Paris Agreement, and the EU climate policy. The main focus of these agreements and policies is on reducing GHG emissions, but the carbon content in the atmosphere can also be offset by carbon sink enhancement (Gren & Aklilu, 2016). Forest carbon enhancement provides a low-cost opportunity in climate policy, but needs effective policy design to be implemented (Gren & Aklilu, 2016). A majority of these carbon sink offset projects have been incorporated in different voluntary systems, in particular under the Reducing Emissions from Deforestation and forest Degradation (REDD) program, which was created by the United Nations in 2008 to increase the use of carbon sinks (UNFCCC, 2008). REDD incentivizes a break from historic trends of increasing deforestation rates and greenhouse gases emissions. It is a framework through which developing countries are rewarded financially for any emissions reductions achieved associated with a decrease in the conversion of forests to other types of land uses (Parker et al., 2009). A country that takes action to effectively reduce rates of deforestation and degradation will be financially rewarded relative to the extent of their achieved emissions reductions (Transparency International, 2012). By economically valuing the role forest ecosystems play in carbon capture and storage, it allows intact forests to compete with historically more financially beneficial, alternate land uses that result in their destruction (Parker et al., 2009).

Ecuador has been a participant in the UN-REDD program since Oct 2009 when it was formally accepted as an observer country. As a megadiverse country that has dealt with large amount of deforestation, it is vital that the country participates in carbon programs like REDD+. There are 91 ecosystems in Ecuador, which cover 15,333.56 hectares, 59.8% of the national territory (The REDD Desk). Historically, Ecuador has been poorly equipped to monitor deforestation and forest degradation; national and regional data on forests are largely outdated, unreliable, incomplete and inconsistent and the current administration has subsequently made great effort and investment to create reliable data on the state of the nation's forests, the first results of which were published in 2011 (MAE, 2011). The agrarian reform that took place during the 1960s and 70s drove thousands of peasant farmers to occupy forested areas in the northwest and the Amazon regions of Ecuador, causing large-scale deforestation and destruction to forests (Morales et al. 2010) As mentioned before, since the acceptance of its National Joint Program (NJP) in March 2011, Ecuador became a beneficiary country and joined the group of twelve pilot countries that are helping to implement activities in preparation for REDD (The REDD Desk).

The first step in completing a REDD project involves establishing the amount of carbon stored within a defined area of the reserve (i.e. carbon stored per hectare of primary forest). The next step involved determining the rate of deforestation that is currently occurring and the amount of deforestation that might be expected if degradation and deforestation activities were to occur within the Reserve. Because Río Guajalito is a protected reserve of 795 acres of mainly primary forest, it is likely not a spot that would be a candidate for a REDD project. However, much of the surrounding area has been affected by deforestation for cattle farming so the calculations found within the reserve could serve as of use for surrounding areas.

Most carbon-offset programs involve a buyer and a seller. One uncertainty common to both the buyer and seller of carbon credits is the variability in weather conditions which affects biomass growth and thereby carbon sequestration in aboveground and belowground living biomass. Another is the uncertainty created by errors in measuring, monitoring, and verifying carbon sequestration. A third uncertainty factor relates to permanence in a created sink, which can be turned into a source through natural events such as wildfires, storms, and insect and pathogen outbreaks (Gren & Aklilu, 2016). The variability in weather condition certainly played a part in the research of this project. Had it not been rainy the majority of the time, biomass calculations may have been different; therefore resulting in different carbon stock measurements.

According to the national greenhouse gas (GHG) inventory for the Intergovernmental Panel on Climate Change (IPCC) sectors, Ecuador's emissions in 2010 were 71.8 million t/CO2eq. These numbers are relatively low when compared to global emissions of 49 billion t/CO2eq, making Ecuador's emissions approximately 0.15% of the world's emissions. Out of this total, the Energy (50%) and Agriculture, Forestry and other Land Uses (43%) sectors are the largest contributors to the country's emissions (UNFCC). Yet Ecuador has still committed itself to reduce its GHG emissions and try to improve its deforestation rates with programs such as the Socio Bosque Program, which is a REDD initiative under the Ministry of the Environment that began in September 2008 (REDD Desk, 2011). The Socio Program has three objectives, which are:

- 1. "Conserve native forests and other native ecosystems to protect their tremendous ecological, economic, cultural and spiritual values. The goal is to conserve 4 million ha of forest and other native ecosystems over the next seven years.
- 2. Significantly reduce deforestation and associated GHG emissions.
- 3. Improve the well-being of farmers, indigenous communities and other groups living in the country's rural areas with the hope to benefit between 500,000 and one million people" (REDD Desk, 2011).

The program consists of direct payments made to the landowners, with the economic incentive varying according to the size of the area that each owner voluntarily enters into the program, with a maximum payment of US\$ 30 per hectare (MAE, 2011). This program would not apply to a reserve like Río Guajalito because there are no farmers or indigenous communities on the reserve, but for areas surrounding the reserve it could be function as a way to discourage deforestation and degradation without finding investors to buy land to conserve like in Río Guajalito.

As mentioned previously, although Río Guajalito would not qualify as a REDD+ site, researching REDD+ was an important part of this study, as above ground carbon stock measurements are the primary concern within a project area (VCS, 2008). A complete REDD+ analysis could not be completed on the site because belowground biomass was not measured, aboveground biomass was not measured in full completeness and deforestation estimates were not calculated. For measuring REDD, countries need to know: "(1) the aerial extent of deforestation and forest degradation (hectares), (2) for degradation, the proportion of forest biomass lost (percentage), (3) where the deforest type (metric tons of carbon per hectare), and (5) the process of forest loss which affects the rate and timing of emissions" (Ramankutty et al 2007).

Suggestions for Future Studies

If this study were to be repeated, it would be helpful to repeat it in a different season with either more or less precipitation. Because biomass in the secondary 2001 and primary forests was very wet from rain, it's possible that it could have skewed weights and carbon measurements. It would be interesting to compare weights of biomass when there wasn't as much rain in the forest. Another suggestion would be to try to make more plots in each type of forest. Because each plot was only 10 m x 10 m, it would it be interesting to compare multiple plots within the same type of forest; this would also allow for more accurate carbon sequestration calculations. Slope and elevation were two components that were used in almost every study during research; therefore; using these variables as a comparison in the same types of forest would be very useful when comparing carbon stock measurements.

Another suggestion for future studies would be to categorize the biomass into more

categories, not just ground litter and large branches. In the Oxford/ GEM methodology used in this study they suggest separating the biomass into more categories, such as soil, leaves, roots and dead wood. Because in tropical forests approximately 50 percent of the carbon is stored in the biomass and 50 percent in the soil (IPCC, 2000), this research should therefore be more or less missing 50% of the carbon stock in each type of forest studied. This is most likely the reason for why when compared to other studies, the amount of carbon stock measured in t C ha⁻¹ is low. To calculate soil carbon stock more time would need to be allotted and more sophisticated tools would need to be used. Also access to a lab would be necessary to analyze soil carbon contents.

Finally, because the Oxford/ Global Ecosystems Monitoring Network (GEM) methodology allows for different plot sizes, it would be interesting to see this study done in larger plots. If larger plots were used, then more tree data could have been gathered and this could have been very useful for data analysis and looking at overall carbon sequestration by trees in each type of forest. The Oxford/ Global Ecosystems Monitoring Network (GEM) methodology also suggests using litter traps for fallen litter in between collection times. This could have been useful for this study because sub-plots surveyed earlier in the week sometimes had a significant amount of extra litter in them due to rain/ movement of the litter.

Limitations

Scraping the forest floor for every piece of organic matter was extremely difficult in the secondary forest because large roots were often in the way. It would have helped had their been another person working at the same time to monitor that all the organic matter had been collected in every sub quadrat. This was not as much of an issue in primary forest and the secondary forest from 2001, as it was very clear when the entire organic layer of soil had been collected and the next layer of soil had been reached. It may have been easier to collect biomass in the primary and secondary forest from 2001 because it started to rain the last two weeks of data collection. Had the secondary forest from 1983 been completed a different week, then it may have been both easier to collect biomass and measurements may have differed because the biomass would have been wet, not dry.

Because there was not enough time to use string throughout the whole plot and measure out every 50 cm x 50 cm sub quadrat, it is possible that each randomized sub quadrat chosen was not exactly 50 cm x 50 cm and was not exactly in the correct location. Next time it would be helpful to make a 50 cm x 50 cm PVC pipe square and place it on the ground instead of using markers at each corner of the sub-plots and measuring in from the perimeter of the 10 m x 10 m plot.

In the secondary forest plot from 1983, 4 out of 7 mornings the plot was set up for work it appeared as if a large animal had walked through it and gotten caught in the string. One morning the string was found 10 meters down the trail from the start of the plot. This meant re-measuring the plot to some extent and perhaps causing some error in measuring the 50 cm x 50 cm sub quadrats. Next time it would be a better idea to make sure the string is tied as tight as possible at every corner and every corner was securely pushed into the ground.

Another possibility of potential error was during collecting samples of trees for density measurements. For some trees collecting a wood sample was virtually impossible with the 4-meter long poles. Sometimes, trees that were nearby of the same species and smaller were used.

It is possible that the other trees weren't the exact same species and therefore could have skewed the density data. This was only one component of the allometric equation used for tree biomass but still could have had some affect on the data. Also, there are many different allometric equations used when estimating carbon stock. If the study had used a different type of allometric equation for tree carbon content the carbon measurements could have been different.

Conclusions

It is important to conserve not just primary forests in cloud forest ecosystems, but secondary forests as well. Although the data from this study does not correlate with others done in similar areas, due to the fact that the secondary forest from 1983 sequesters more carbon than the primary forest, there is plentiful evidence that secondary forests are also hotspots for diversity and have the potential to sequester considerable amounts of carbon. Biomass storage in secondary tropical forests returns to pre-disturbance values after 80 years (Martin et al., 2013); so if these forests are cut down for logging, petroleum extraction or agriculture they do not have a chance to return to sequestering large amounts of carbon. The weight of the wet biomass does not necessarily correlate with the amount of carbon sequestered by a forest. Tree distribution/ diversity data was fairly inconclusive because not enough plots could be surveyed to get more comparative data. Because the secondary forest from 1983 had the most trees it appeared as if the trees there sequestered the most carbon and also had the highest Shannon Diversity Index numbers.

Although this data is just the first step to start a REDD+ project in the area and the reserve would not qualify as a REDD+ site, seeing above ground biomass carbon stock measurements is a step in the right direction to conserving more surrounding forest areas without turning them into official reserves like Río Guajalito. Altogether, avoided forest degradation, avoided deforestation, and forest regeneration and restoration could stabilize the atmospheric concentration of CO2 while fossil fuels are replaced by renewable fuels over the next few decades (Houghton et al, 2015), thereby providing a reasonable chance of limiting global warming to less than 2°C.

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Appendix

Wet Biomass Weights (Appendix 1)

	Ground Litter Total (kg)	Branches Total (kg)
Secondary Forest- 1983	90.78	24.68
Primary Forest	130.18	78.16
Secondary Forest- 2001	116.23	21.58

Dry Biomass Weights (weighed after representative 1 kilogram sample was dried for 48 hours at 70°C) (Appendix 2)

	Ground Litter Total (g)	Branches Total (g)
Secondary Forest- 1983	530	560
Primary Forest	250	270
Secondary Forest- 2001	200	225

Tree Data

Secondary Forest- 1983 (Appendix 3)

Tree	DBH (cm)	Height (m)	Density (g/mL)	Biomass (t ha ⁻¹)
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Palicourea	3	1.65	0.87	0.63
Miconia	8.1	4.89	1	5.78
Miconia	3.9	1.3	1	0.74
Clusia pseudomangle	8.5	4.5	1	5.58
Dead- unidentified	23.5	3.26	0.32	3.58
Clusia pseudomangle	33.4	14	0.6	40.93
Miconia	9.2	5.92	0.94	7.47
Dead- unidentified	22.7	10.3	0.33	11.26

Primary Forest (Appendix 4)

Tree	DBH (cm)	Height (m)	Density (g/mL)	Biomass (t ha ⁻¹)
Psychotria	5	2.26	1	1.65
Miconia	15.1	6	0.94	12.42
Miconia	8.3	7.9	0.36	3.44
Styrax cf. cordatus	27.9	7.41	0.21	6.33
Nectandra reticulata	4	2.35	1	1.37
Eschweilera caudiculata	4.6	5.57	1	3.74
Palicourea demissa	3.9	2.4	1	1.37

Secondary Forest- 2001 (Appendix 5)

Tree	DBH (cm)	Height (m)	Density (g/mL)	Biomass (t ha ⁻¹)
Exarata chocoensis	13.5	5.93	1	11.68
Cyathea	10.9	1.4	0.95	2.11

Weinmannia macrophylla	40.3	8.3	0.5	29.69
Cyatheae	18.8	5.15	0.95	13.42
Miconia	7.4	3.1	1	3.35
Dead- unidentified	7.5	1.9	0.79	1.64