Regional Spatial Variation in Aboveground Biomass, Forest Structure and Species Composition of Bolivian Tropical Dry Forests

A Remote Sensing and Field-Based Analysis



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October 2015

Master Thesis MSc programme Sustainable Development Specialization: Global Change & Ecosystems Utrecht University, the Netherlands

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Index

Acknowledgements	
Abstract	4
Resumen	5
Acronyms	6
1. Introduction	7
1.1 Climate Change, Tropical Forests and Deforestation	7
1.2 Reducing Emissions from Deforestation and Forest Degradation	
1.3 REDD+ in Bolivia	8
1.4 Fundación Natura Bolivia	9
1.5 Quantifying Avoided Deforestation	10
1.6 Tropical Dry Forest	10
1.7 Measuring Carbon Stocks	11
1.7.1 Field-Based Measurements	12
1.7.2 Remote Sensing	13
1.8 Spatial Variation in Aboveground Biomass and Forest Structure	14
1.9 Spatial Variation in Plant Species Composition	15
1.10 Problem Definition and Research Aim	16
1.11 Research Question and Hypotheses	17
2. Methodology	18
2.1 Study Area	
2.2 Remote Sensing Data	21
2.3 Field-Based Measurements	23
2.4 Calculations of Aboveground Biomass	28
2.5 Collection of Environmental Data	30
2.6 Data Analysis	30
3. Results	34
3.1 Effects of Methodological Choices	34
3.1.1 Effect of Allometric Equation	
3.1.2 Effect of Plot Size	35
3.1.3 Effect of Lower DBH Limit	
3.2 Analysis of Spatial Variation	
3.2.1 Spatial Variation in Aboveground Biomass	
3.2.2 Biomass Distribution across Forest Compartments	41
3.2.3 Spatial Variation in Forest Structure	
3.2.4 Spatial Variation in Species Composition	
3.3 Multivariate and Regression Analysis	49
3.3.1 Multivariate Ordination Analysis of Species Composition and Diversity	49
3.3.2 Multivariable Regression Analysis of Aboveground Biomass	54
3.4 Mapping Aboveground Biomass in the ANMI RG-VC	55

4. Discussion	59
4.1 Comparison of Obtained AGB Values	59
4.2 Sources of Error	60
4.3 Ecoregion and Ecosystem Averages	63
4.4 Modelling Aboveground Biomass	63
4.5 Forest Structure	64
4.6 Species Composition	65
4.7 Linking Species Composition, Forest Structure and Biomass	66
5. Conclusion	67
References	69
Appendix	78

Acknowledgements

This Master thesis research project was carried out in the form of a research internship at the Bolivian non-governmental organization Fundación Natura Bolivia (FNB). I am very grateful for the opportunity that FNB gave me to conduct this research at their organization. I specifically want to thank Tito Vidaurre for the research idea, as well as his support and supervision throughout my time in Bolivia. I wish to thank Lucindo Gonzáles for his organizational and logistical support in setting up the various field campaigns. I also wish to thank Veronica Chavez and all the other FBN staff for their continuing help and friendship during my stay with them. I learned a lot from them all.

Most specifically, I want to extend my gratitude to Ivan Linneo, who accompanied me on each single field trip. He was an indispensable source of knowledge and energy, both in the field as well as during the process of botanical identification and analysis. His tireless enthusiasm and dedication to his work was truly inspiring.

I also wish to thank Paola Luna, Edson Cortez and John Mendoza for their friendship and continuing support throughout our fieldtrips together. Without their work, there would have been no data to report on in this thesis.

Furthermore, I want to thank all the local farmers and residents that allowed us to work and camp on their lands, namely Don Angel Avila in Chiriguanañan, Don Epifanio Vivero in Algodonales, Don Carlos Avalos and Don Saul Albarto in Molleaguada, Don Reinaldo Escobar in Salsipuedes Grande, Don Paulino and Doña Maria Seas in Laja Toco, Don Flabio in Pampa Negra, Don Benedicto Tupuri in Bicoquin, and Don Aurelio in Tocopampa. They have shown me a generous hospitality for which I am greatly indebted.

Lastly, I want to thank my supervisor Jerry van Dijk for his constructive support throughout the project. His critical ideas and feedback have helped me a lot in conducting this research and writing up this report.

Abstract

Tropical forest deforestation is a major source of global anthropogenic carbon emissions. Consequently, preventing deforestation via REDD+ has lately gained much attention. In Bolivia, Fundación Natura Bolivia has set up local forest conservation schemes in the ANMI RG-VC area, which could be used for future application towards REDD+. However, regional baseline maps of biomass distributions are much required for this. The ANMI RG-VC area is a topographically and ecologically heterogeneous terrain at Bolivia's Andes-to-Amazon transition zone. Its vegetation can largely be characterized as tropical dry forest, but aboveground biomass (AGB), forest structure and species composition are known to show large spatial variation. Hence, the research question of this research was: What is the influence of environmental factors on regional AGB estimates for avoided deforestation projects in the tropical dry forests of the ANMI RG-VC, and how is this related to forest structure and species composition? This was addressed by setting up a factorial stratified sampling design across two factors: forest type class and elevation. Forest type class was defined as a combination of one of the main ecoregions (Tucuman-Bolivian forest, Inter-Andean Dry forest and Chaco Serrano) with a forest cover classification (evergreen or deciduous forest). 21 0.1 ha (20x50m) forest plots were installed across 8 sites for measurements of AGB, forest structure and species composition. Methodological choices were addressed by analysing the effect of allometric equations, plot size and lower DBH limits. Landscape variability was assessed via multivariate ordination and regression analyses. It was found that forest cover type did not cause any significant differences in AGB. Hence, spatial variations in AGB were assessed at ecoregion and ecosystem level only. This resulted in an estimated 108 or 101 Mt AGB for the entire region, or on average 179 and 166 t/ha of forest, respectively. The allometric equation by Brown (1997) was found to be the most reliable, while plot size or lower DBH limit did not significantly affect the results. AGB was found to be unrelated to elevation, but strongly related to drought stress (between ecoregions), or climatic water deficit (within Tucuman-Bolivian forest). Species composition between ecoregions was related to drought stress as well, but within Tucuman-Bolivian forest, elevation turned out to be the strongest predictor variable. Hence, a differential environmental effect on AGB and species composition was found for this ecoregion. It is thought that this is because stem density and basal area mediate the effect of species composition on biomass.

Resumen

La deforestación de bosques tropicales es una de las principales fuentes de las emisiones globales antropogénicas de carbono. En consecuencia, la prevención de la deforestación a través de REDD+ ha ganado recientemente mucha atención. En Bolivia, la Fundación Natura Bolivia ha establecido proyectos locales de conservación de los bosques en la región ANMI RG-VC, que podrían utilizarse para la futura aplicación hacia REDD+. Sin embargo, se requieren mapas de línea de base de las distribuciones regionales de biomasa área para esto. La región ANMI RG-VC es una zona topográficamente y ecológicamente muy heterogénea, situado en la zona Boliviana transicional de Andes-a-Amazonia. Su vegetación en gran parte puede ser caracterizada como bosque seco tropical, pero la biomasa aérea (AGB), la estructura del bosque y la composición de las especies son conocidas para mostrar gran variación espacial. Por lo tanto, la pregunta de esta investigación fue: ¿Cuál es la influencia de los factores ambientales en las estimaciones regionales de AGB para proyectos de deforestación evitada en los bosques secos tropicales del ANMI RG-VC, y cómo se relaciona esto con la estructura del bosque y la composición de las especies? Esto se abordó mediante la creación de un diseño de muestreo estratificado factorial con dos factores: la clase de tipo forestal y la elevación. Clase de tipo forestal se define como una combinación de una de las principales ecorregiones (bosque Tucumano-Boliviano, bosques secos interandinos y Chaco Serrano) con una clasificación de la cubierta forestal (siempre verde o caducifolio). 21 0.1 ha (20x50m) parcelas de muestreo se instalaron en 8 sitios para las mediciones de AGB, la estructura del bosque y la composición de las especies. Opciones metodológicas fueron abordados mediante el análisis del efecto de las ecuaciones alométricas, tamaño de la parcela y el límite inferior de DAP. La variabilidad del paisaje se evaluó mediante análisis de ordenación multivariante y de regresión. Se encontró que el tipo de cubierta forestal no causó ninguna diferencia significativa en AGB. Por lo tanto, las variaciones espaciales en AGB fueron evaluados solo al nivel de las ecorregiones y ecosistemas. Esto resultó en un estimado de 108 o 101 Mt AGB para toda la región, o en promedio 179 y 166 toneladas de hectárea de bosque, respectivamente. Se encontró que la ecuación alométrica por Brown (1997) era el más confiable, mientras que el tamaño de parcela o el límite inferior de DAP no afectaron significativamente los resultados. Se resultó que AGB no está relacionado con la elevación, pero está fuertemente relacionada con el estrés por seguía (entre ecorregiones), o déficit hídrico climático (climatic water deficit, dentro de los bosques Tucumano-Boliviano). La composición de especies entre las ecorregiones estaba relacionada con el estrés por sequía también, pero dentro de los bosques Tucumano-Boliviano, elevación resultó ser la variable predictora más fuerte. Por lo tanto, se encontró un efecto ambiental diferencial entre la composición de especies y AGB para esta ecorregión. Según parece esto se debe a que la densidad de tallos y el área basal median el efecto de la composición de especies sobre la biomasa área.

Acronyms

Aboveground biomass
Aboveground live biomass
the Río Grande – Valles Cruceños Natural Area of Integrated Management
(Área Natural de Manejo Integrado Río Gande - Valles Cruceños)
Canonical correlation analysis
Chaco Serrano forest
Climatic water deficit (in mm/yr)
Diameter at breast height (~1.30 m)
Detrended correspondence analysis
Digital elevation model
Food and Agricultural Organization of the United Nations
Fundación Natura Bolivia
Greenhouse gas
Generalized linear model
Inter-Andean Dry forest
Intergovernmental Panel on Climate Change
Biomass-adjusted Importance Value Index
Mean wood density (in g/cm ³)
Normalized difference vegetation index
Average total precipitation of the driest quarter (in mm)
Payment for ecosystem services
Precipitation seasonality (coefficient of variation)
Redundancy analysis
Reciprocal environmental agreement
Reducing emissions from deforestation and forest degradation
Reducing emissions from deforestation and forest degradation in developing
countries (including conservation, sustainable management and
enhancement of carbon stocks)
Remote sensing
Reciprocal watershed agreement
Tucuman-Bolivian forest
Tropical dry forest

1. Introduction

1.1 Climate Change, Tropical Forests and Deforestation

Global anthropogenic climate change is one of the most pressing issues of the 21st century. Rockström *et al.* (2009) identified climate change as one of three important planetary boundaries that have already been transgressed due to anthropogenic impact. According to the most recent assessment report by the Intergovernmental Panel on Climate Change, the increase in anthropogenic greenhouse gas (GHG) emissions (primarily carbon dioxide, methane and nitrous oxide) since the beginning of the industrial area is "extremely likely to have been the dominant cause" of observed global warming (IPCC 2014). Of the various greenhouse gases, carbon dioxide is the single largest contributor to these effects (IPCC 2014).

One of the causes of this increase in carbon dioxide (CO₂) emissions is large-scale land-use change (Malhi and Grace 2000). According to Rockström *et al.* (2009), we may reach a point where "further agricultural land expansion at a global scale may seriously threaten biodiversity and undermine regulatory capacities of the Earth System". Especially in the tropics, land-use changes are severe and can have pronounced climatic and environmental effects (Malhi & Grace 2000; Foley *et al.* 2005). Tropical forests are inextricably linked to global climate by storing and regulating large quantities of carbon, as well as through hydrological feedback loops and surface albedo (Cramer *et al.* 2004; Foley *et al.* 2005; Chow *et al.* 2013). In terms of land surface, tropical forests cover a relatively small part of Earth's total terrestrial surface of only 17 per cent (Buchmann *et al.* 1997). 40 per cent of this can be found in the Amazon region (Verweij *et al.* 2009). However, within the global carbon system, tropical forest are considered one of the most important carbon sinks, as they store approximately 40 per cent of all carbon in terrestrial vegetation (Baraloto *et al.* 2007), out of a globally estimated tropical forest biomass of 229 Pg C (Baccini *et al.* 2012). Furthermore, with an average of 2.8 Pg C year⁻¹, tropical forests account for about 70 per cent of the gross C sink in the world's forests (Pan *et al.* 2011),

However, as Pan *et al.* (2011) point out as well, tropical forest are also the biggest forest carbon source due to land-use changes, with an estimated emission of 1.3 Pg C year⁻¹. This consists of a gross tropical deforestation emission of 2.9 Pg C year⁻¹, which is compensated by a carbon sink due to tropical forest regrowth of 1.6 Pg C year⁻¹. Recent insights, however, suggest the Amazonian carbon sink capacity is declining (Brienen *et al.* 2015), which could further shift the sink-source balance of tropical forests towards the latter. In sum, tropical forests account for the largest stocks of all forests within the carbon cycle, but also for the largest flows due to intense land-use change (Pan *et al.* 2011).

This land-use change often takes the form of deforestation or forest degradation. In recent estimates by Achard *et al.* (2014), gross loss of tropical forest cover due to land-use changes was estimated at 8.0 million ha year⁻¹ in the 1990s and 7.6 million ha year⁻¹ in the 2000s (0.49 per cent annual rate). In its most recent Global Forest Resource Assessment, the Food and Agricultural Organization estimated that the global loss of forest cover declined from 8.5 million ha year⁻¹ in the 1990s to 6.6 million ha year⁻¹ in the period 2010-2015 (FAO 2015a). The resulting carbon losses from this forest loss were estimated at 0.89 and 0.88 Pg C year⁻¹ in the 90's and 2000's, respectively (Achard *et al.* 2014). Baccini *et al.* (2012) estimated the net C emission from tropical deforestation and land use changes over the

same period 2000-2010 to be 1.0 Pg C year⁻¹. Because of this, tropical deforestation and forest degradation represents the second largest source of global GHG emissions, accounting for 12-20 per cent (Ghazoul *et al.* 2010) or 12-15 per cent (Kotowska *et al.* 2015) of global anthropogenic carbon emissions, or 6-17 per cent of global anthropogenic CO₂ emissions (Baccini *et al.* 2012). Similarly, Achard *et al.* (2014) report that the C losses from deforestation are roughly 10 per cent of other anthropogenic C emissions, such as those due to fossil fuel combustion or cement production. Thus, although the exact figure varies, it is clear that carbon emissions form deforestation or forest degradation are important contributors to global anthropogenic climate change (IPCC 2014). Furthermore, it is generally recognized that large-scale land cover changes also have negative regional and local effects, such as loss of biodiversity or increased erosion (Brook *et al.* 2003; Fearnside 2005; Salimon *et al.* 2011).

1.2 Reducing Emissions from Deforestation and Forest Degradation

From the figures above, it is clear that the current loss of tropical forests is substantial and has severe environmental impact. For this reason, there is an increasing need to prevent further deforestation as much as possible and conserve the remaining tropical forests. One way to do so has been by setting up 'Payments for Ecosystem Services' (PES) schemes. These schemes are market-based mechanisms in which governments or non-governmental organizations pay for public environmental services, such as watershed protection or carbon sequestration. As has been stressed by Kinzig et al. (2011), these mechanisms promise much, but are often poorly designed or implemented, in which case they can make things worse (Ghazoul et al. 2010). Within this framework, carbon storage or sequestration is viewed as a regulating service that becomes increasingly important in light of global climate change (Paruelo & Vallejos 2013). One specific and prominent PES scheme for carbon sequestration is REDD (Reducing Emissions from Deforestation and Forest Degradation). In recent years, this has been extended into REDD+, in which conservation, sustainable management, and enhancement of forest carbon stocks in developing countries is taken into account as well (Venter & Koh 2012). The main idea of REDD+ is that the reduction of carbon emissions from deforestation and forest degradation below an expected reference level provides 'additional' ecosystem services, which is worth an amount of carbon credits on the international market. In this way, REDD+ provides a financial incentive to curb deforestation rates (Agrawal et al. 2011; Hall 2012; Venter & Koh 2012).

1.3 REDD+ in Bolivia

When it comes to deforestation and REDD+, Bolivia is a relevant country to study. Tree cover in Bolivia is 53 per cent of the country's total area (57 million ha), of which two-thirds is primary forest. Deforestation rates are high and have been rising. In the 1990s, the estimated annual deforestation rate was 0.44 per cent (Hall 2012), or on average 270 333 hectares per year between 1993 and 2000 (FAO 2015b). Between 2005 and 2010, that deforestation rate had risen to 0.53 per cent annually, which is one of the highest figures worldwide (Hall 2012). The FAO (2015b) now reported an average forest loss of 302 249 hectares per year over the period 2004-2007. In the twenty years between 1990 and 2010, Bolivia's total forest cover decreased with almost 9 per cent from 62.8 million hectares to 57.2 million hectares (FAO 2015b). Consequently, 80 per cent of Bolivia's greenhouse gas emissions are caused by forest loss or degradation, which is thought to be the highest proportion in Latin-America (Hall 2012). Three quarters of this deforestation occurs in the Department of Santa Cruz, which covers

the eastern lowland and harbours vast areas of Bolivian Amazon forest. The main causes of this deforestation are the expansion of commercial farming and settler agriculture in these lowland areas, as well as illegal logging (Hall 2012).

Since Bolivia has high forest cover and high deforestation rates, the country is believed to be very suitable for implementation of REDD+ projects. Because of this, Bolivia has several years of experience with PES schemes, and developed one of the first REDD projects worldwide, the famous Noel Kempff Climate Action Project (Brown *et al.* 2000; Hall 2012). Implementation of these schemes has proven rather difficult though, with the greatest challenges being the slow process of building trust between service buyers and providers, as well as in achieving clear environmental additionality provided by these ecological services (Asquith *et al.* 2008).

However, since 2010, under left-wing president Evo Morales, Bolivia has publicly opposed the idea of REDD+, for two reasons: because it represents a commodification of nature, and because it was seen as transferring a burden of responsibilities from developed to developing countries (GCF 2015). Since then, Bolivia has been trying to develop an alternative, non-market based approach to REDD+. This resulted in the locally developed Joint Mitigation and Adaptation Mechanism for the Comprehensive and Sustainable Management of Forest and the Mother Earth (MMAyA 2012). According to the Global Canopy Foundation, the main features of this mechanism are a recognition of the dual importance of forests for both mitigation and adaptation, and a focus on the non-commodification of forests (GCF 2015).

1.4 Fundación Natura Bolivia

One way in which the Joint Mitigation and Adaptation Mechanism has been implemented locally in Bolivia is in the form of so-called Reciprocal Environmental Agreements developed by Fundación Natura Bolivia (FNB). Fundación Natura Bolivia is a Bolivian non-governmental organization, established in 2003. It aims to help local communities in Bolivia protect their own water sources through the conservation of their forests. For this, the foundation has set up local schemes called Reciprocal Environmental Agreements (REA, or ARA in Spanish). Reciprocal Environmental Agreements are an extension of initial Reciprocal Watershed Agreements (RWA). Originally, the foundation's focus was on preserving water sources. For this, the foundation set up a mechanism in which downstream water users contribute to a financial fund for upstream forest conservation. Because upstream forests are vital for conserving water, it is very important to conserve these forest if one wish to obtain sustained water supply in the future. Downstream users are usually the municipal government and local water users who are depended on the upstream water source. This might include agricultural companies or multinational corporations as well. They contribute to a conservation fund which is used to support the upstream communities in building sustainable livelihoods by preserving forests on their land and finding economically feasible projects in the meantime, such as beekeeping (FNB 2015).

The initial RWAs focused on watershed functions only, but Fundación Natura Bolivia has been expanding these agreements to include other ecosystem services as well, such as carbon sequestration, biodiversity conservation and local climate control. Thus, these mechanisms became Reciprocal Environmental Agreements (FNB 2015).

By 2013, Fundación Natura Bolivia had been involved in setting up REA schemes in 30 municipalities across three regions in the Santa Cruz department of eastern Bolivia: Amboró National Park, the Río Grande - Valles Cruceños Natural Area of Integrated Management, and the El Chaco region. In total, the foundation has 83.725 hectares of tropical forest under conservation, providing financial support to roughly 2000 families (FNB 2015).

1.5 Quantifying Avoided Deforestation

In order to better understand the effectiveness of these schemes in terms of their carbon sequestration service, Fundación Natura Bolivia tries to quantify the amount of avoided deforestation (and as such, avoided GHG emissions). In order to do so, baseline scenarios of land-use change are critically important. Such a baseline scenario consists of projected land-use changes and the corresponding carbon stocks in vegetation and soil (Brown *et al.* 2007). In the case of FNB, a baseline scenario with normal deforestation rates is compared with a conservation scenario in which the REA schemes by FNB are taken into account. For both scenarios, deforestation rates are spatially modelled with Land Change Modeler (Clark Labs 2010). This analysis was recently done for tropical moist forest in the municipality of San Carlos, up to the year 2023 (Maillard 2014). Differences in deforestation between the two scenarios (in hectares) were converted into avoided total emissions of CO₂- equivalent (in tonnes), based on field-based biomass data (in tonnes/ha) obtained in the same municipality (Carreño-Rocabado 2014; Maillard 2014).

1.6 Tropical Dry Forest

However, such biomass data does not yet exist for FNB's main working area: the Río Grande - Valles Cruceños Natural Area of Integrated Management. This area is characterized by a large cover of tropical dry forest. Tropical dry forest (TDF) is different from tropical rain forest in that it experiences a pronounced seasonality in rainfall distribution, resulting in a significant dry season for part of the year (Miles et al. 2006). Under the Holdridge Life Zones Classification, tropical dry forest is defined as a subhumid ecosystem with a mean annual temperature greater than 24 °C, a mean total annual precipitation between 1000 and 2000 mm, and a potential evaporation ratio of 1:2 (the ratio of annual potential evapotranspiration to mean total annual precipitation). This ratio is 2-4 times higher than that of tropical rain forest (Kricher 2011). However, reality is often much more complex, and varies regional and global classifications of TDF exist (Miles et al. 2006). Another common definition is that of the seasonally dry tropical forest (SDTF), which is defined as a tropical forest with an annual precipitation between 250 and 2000 mm and the presence of a strong dry season lasting 3-4 months (Becknell et al. 2012). According to Becknell et al. (2012), SDTFs comprise a globally extensive biome, representing up to as much as 42% of all tropical forests. Since dry forest typically has a high wood density (Chave et al. 2006), these forests are likely to store significant amounts of carbon. However, due to its susceptibility to drought, this type of forest is also likely to be strongly effect by global climate change (Araujo-Murakami et al. 2014), let alone deforestation and a variety of other anthropogenic threats (Miles et al. 2006). For this reason, tropical dry forests are commonly seen as among the most threatened terrestrial ecosystems (Quesada et al. 2009a; Hernández-Stefanoni et al. 2011).

Hence, tropical dry forest is expected to have an important impact on global carbon budgets in the future. Yet to date, this type of forest is comparatively less studied (Hernández-Stefanoni *et al.* 2011;

Becknell *et al.* 2012). Especially, little attention has been given to this type of ecosystem from a global carbon cycling perspective (Araujo-Murakami *et al.* 2014).

Specifically in southeast Bolivia, tropical dry forest covers an extensive area that suffers from a relatively large cover loss: 12 per cent annually, averaged over the whole of Latin-America between 1980 and 2000 (Miles *et al.* 2006). Hence, analysing the effectiveness of local conservation schemes using avoided deforestation baselines is critically important here. Because of this, there is a strong need to obtain a proper baseline of biomass distributions among the tropical dry forests in this area. This data can then be used to study the effect of FNB's conservation schemes on carbon emissions using the land change modelling technique by Maillard (2014).

Yet, however much required, accurately estimating biomass stocks in tropical forests remains a technical challenges (Gibbs *et al.* 2007; Ghazoul *et al.* 2010; Salimon *et al.* 2011). Different methods for estimating and monitoring carbon stocks and fluxes exist, but a scientific consensus on the most accurate technique is still lacking (Gibbs *et al.* 2007).

1.7 Measuring Carbon Stocks

Most carbon in tropical forest ecosystems is stored in the living biomass of trees and understory vegetation, as well as in dead litter mass, woody debris and soil organic matter. The aboveground living biomass (AGLB) of trees generally forms the largest carbon pool and is the one that receives the biggest impact from deforestation (Gibbs et al. 2007). Estimates for AGLB generally range from 65 to 80 per cent of total biomass, depending on the type of ecosystem (Gibbs et al. 2007; Fonseca et al. 2012; Kotowska et al. 2015). Necromass (dead woody biomass) and herbaceous vegetation are often neglected in forest biomass studies, but can constitute up to 15 per cent of total aboveground biomass (AGB) in forests (Fonseca et al. 2012). Gibbs et al. (2007) mentioned a value of 10-20 per cent of total AGB equivalent for necromass as well. Additionally, belowground (root) biomass can take up an extra 20 per cent of total forest biomass (Fonseca et al. 2012). Next to above- and belowground biomass, soil carbon is another important compartment for overall carbon storage. Quantities of soil carbon are hard to measure and vary greatly per region and forest type, but can be similar to or even larger than total aboveground carbon values in the humid tropics (Fonseca et al. 2012). Estimates range from 36 to 60 per cent of total ecosystem carbon (Don et al. 2011). For this reason, soil carbon is another critical carbon compartment to study. However, unlike in cases like Southeast Asia's peat-swamp forests, impacts on soil carbon stocks from tropical deforestation are generally less than on the aboveground biomass compartment (Gibbs et al. 2007). For this reason, estimating aboveground biomass is the most critical step in analysing carbon stocks and fluxes for possible REDD purposes in most of the tropics.

It is generally assumed that 50 per cent of the overall total biomass is made up of actual carbon (Lamlom & Savidge 2003). However, studies have shown that this carbon fraction can vary significantly per plant species (Lamlom & Savidge 2003; Fonseca *et al.* 2012). This is due to plant specifics in chemical composition, anatomy, and as a results, overall wood specific density. Recently, some research is starting to use a more accurate carbon fraction of 47 or 48 per cent of biomass (Gibbs *et al.* 2007; Martin & Thomas 2011; Raich *et al.* 2014). However, variations in the carbon fraction of tropical forests might be large due to strong spatial variations in species composition. These variations in species composition are often related to environmental gradients (Engelbrecht *et al.* 2007), which

can cause large estimation errors on regional scales. Scientific consensus on a proper carbon fraction value is thus still lacking. For this reason, most research only measures aboveground biomass stocks in tonnes per hectare. In this study, the words biomass and carbon stocks are sometimes used interchangeably, but all results are given as biomass values in tonnes per hectare.

Generally, one can identify three main methods for estimating forest biomass stocks (Gibbs *et al.* 2007). The biome-average approach assigns a single biomass values to a certain land unit (tonnes/ha) for broad forest or biome categories, generally on a national scale. One source of data input for this consists of biomass harvest data, for which all trees in a certain area are harvested, dried and weighted. Although this produces very accurate results, this methods is very time-consuming, expensive, ecologically destructive, and very location-biased, which makes its data unrepresentative for larger areas (Gibbs *et al.* 2007). Another source of data for the biome-average approach can be forest inventory data as collected by organizations like the FAO. However, these inventories are generally not designed for extrapolation to a country or biome-scale. As such, this approach produces very questionable results if no representative inventory design for large areas has been used (Gibbs *et al.* 2007). However, two other more recent methods might produce more accurate results. These will be discussed in the next parts.

1.7.1 Field-Based Measurements

One method which is less destructive and often more accurate, is the estimation of AGB using local ground-based forest inventory data. This method relates ground-based measurements of tree diameters or tree height to forest biomass stocks using allometric equations. Allometric equations are regression models to statistically relate these diameter or height values to accurate destructive harvest measurements (Gibbs *et al.* 2007). Often, the development of these allometric models is based on large datasets of a single forest type spanning multiple countries or continents (Chave *et al.* 2005). This means that developing allometric equations is time-consuming and expensive, but once they're developed, they can easily be applied to many cases at relatively low costs (Gibbs *et al.* 2007).

Currently, several allometric equations for tropical forests exist and are used alongside each other in most research. The most common ones are by Brown *et al.* (1989), Brown (1997) and Chave *et al.* (2005, 2014). These are generalized equations that are applicable to forest types as a whole, rather than species-specific equations, since the tropics generally contain a large amount of species per hectare (Chave *et al.* 2005). Still, these models are often developed for specific forest types, rather than for tropical forest in general. The differences in AGB between forest types can be large, and specific models per forest type thus improve the accuracy of the estimates (Chave *et al.* 2005). Generally, a distinction is made between dry, moist and wet tropical forest (Brown *et al.* 1989; Brown 1997; Chave *et al.* 2005). Alvarez *et al.* (2012) argued that a forest classification based on Holdridge's Life Zone system systematically produces the best estimates. However, the most recent allometric equations for tropical forests by Chave *et al.* (2014) are designed as pantropical equations and seem to produce accurate results as well.

The input data for these allometric models is obtained from forest plots according to standardized sampling methods. The most common forest plot inventory method is the 1 ha forest plot as first recommended by the FAO in 1981 (Baraloto *et al.* 2013). However, many other methods have since been proposed and no standard currently exists. Baraloto *et al.* (2013) reported that several smaller

(<1 ha) plots can reduce the variation in estimated AGB, but at the same time increase the human effort required. However, they caution against the use of another popular method, the so-called 0.1 ha Gentry plot, as these can be susceptible to a large degree of error (Baraloto *et al.* 2013). On the other hand, many smaller plots can be more suitable for spatially heterogeneous forests. It is an important criterion for the selection of plot location that the plots should encompass a homogenous and representative forest structure of the area for which an estimate is desired. Furthermore, it needs to be taken into account whether static (e.g. carbon stock) or dynamic (e.g. carbon flow) forest data is desired. Many transects and smaller plots are often not permanent and therefore less suitable for dynamic descriptors (Baraloto *et al.* 2013). Additionally, the choice of method also depends on the available time and financial resources, i.e. the method's cost-efficiency (Köhl *et al.* 2011).

Uncertainties related to methodological choices are large. According to Wagner *et al.* (2010), more than 90 per cent of variability in forest descriptors like AGB was based on plot size and census interval (in the case of dynamic data). Chave *et al.* (2004) quantified the statistical error of four uncertainty types: 1) error due to tree measurement, 2) error due to choice of allometric model, 3) sampling uncertainty (plot size), and 4) representativeness of a network of small plots across a vast forest landscape. They found that the greatest source of error is the choice of allometric model (type 2), which accounts for about 10 per cent uncertainty of the mean. This confirms a similar conclusion by Keller *et al.* (2001). Pan-tropical models seem to be the most accurate, as those are often based on larger datasets. Furthermore, the models should be applied only for trees with diameters that fall within the range for which these models are designed (Chave *et al.* 2004). Error type 4 was found to be large as well, specifically in heterogeneous landscapes with strong environmental gradients (Keith *et al.* 2010; Chave *et al.* 2004). In order to prevent this, Chave *et al.* (2004) recommend to use at least a total of 5 ha of forest plots for a landscape-scale AGB estimate. Furthermore, in order to reduce error type 3, plots should measure at least 0.25 ha in size. This latter error accounts for about 10 per cent uncertainty of the mean as well, but this can be reduced with larger plot size.

Measurement error (type 1) generally accounts for only a very small fraction of total error. However, this might not be the case for the estimation of tree height when this parameter is included in the allometric models. According to Hunter *et al.* (2013), the error in tree height estimates results in a 5-6 per cent uncertainty in biomass on a 1 ha-scale. Feldpausch *et al.* (2012) showed that tree height is an important allometric factor that needs to be taken into account to reduce type 2 errors. They argued that tree height can best be modelled allometrically based on tree diameter.

1.7.2 Remote Sensing

More recently, though, remote sensing (RS) techniques have become increasingly popular as an alternative or supplement to field-based methods (Pettorelli *et al.* 2014). Remote sensing involves data acquired from sensors on satellite or airborne platforms (De Fries *et al.* 2007). Currently, this is the only way for obtaining nation-wide base maps of forest biomass. New remote sensing techniques are now able to directly produce AGB stocks without the need for field-based data (Goetz & Dubayah 2011). However, these methods are expensive and not yet operational on a large scale. Furthermore, various research has shown that there are markedly divergent estimates between field-based methods and these remote sensing methods (Hill *et al.* 2013; Mitchard *et al.* 2014). This shows that although many improvements have been made, there is still much to gain in reliably estimating aboveground biomass. Currently, the most reliable estimates can probably only be obtained by combining remote

sensing techniques with field-based data (De Fries *et al.* 2007; Gibbs *et al.* 2007). This 'ground-truthing' of remote sensing data combines the local biomass values in tonnes per unit area with high resolution RS data on forest cover and type. This can be used to produce regional or national estimates with medium to high confidence (De Fries *et al.* 2007).

1.8 Spatial Variation in Aboveground Biomass and Forest Structure

Correctly assessing AGB stocks for this ground-truthing of RS data has lately received much attention within ecology (Saatchi *et al.* 2011). Tropical forests landscapes are often a mosaic of various forest types and of forests in various stages of succession. This results in spatial variation of biomass as well. These variations need to be taken into account when using remote sensing data for regional or nationwide extrapolation (Barbosa *et al.* 2014). This involves identifying spatial variation of ecological processes and understanding the relative influence of environmental gradients on a landscape-scale.

Spatial variation in aboveground biomass is generally caused by multiple of these environmental gradients. On a global scale, one can identify different biomes as the most important variation, because biomes incorporate the major bioclimatic gradients such as temperature, precipitation and geologic substrate (Gibbs *et al.* 2007). Furthermore, AGB can vary locally because of slope, elevation, drainage, soil type or land-use history (Gibbs *et al.* 2007).

However, even within distinct global biomes such as tropical dry forest, bioclimatic gradients cause the largest regional variation in AGB. Becknell *et al.* (2012) found that mean annual precipitation alone explained more than half of the total AGB variation within seasonally dry tropical forests. This strong influence of precipitation is likely to explain why many studies have found a specific east-west geographic gradient in AGB across the Amazon basin. Central and eastern Amazon forests are consistently reported to have overall higher AGB values (>300 tonnes/ha) than the drier western periphery (<300 tonnes/ha) (Malhi *et al.* 2006; Saatchi *et al.* 2007, 2011; Baraloto *et al.* 2011). Specifically, Saatchi *et al.* (2007) found that AGB shows a high spatial variation which is not directly related to vegetation type. Rather a significant correlation with the length of dry season was found. This confirms a similar negative correlation found by Chave *et al.* (2004) between dry season and total AGB. It suggest that biomass accumulation in tropical forests is most limited by water availability throughout the dry season (Saatchi *et al.* 2007).

According to Malhi *et al.* (2006) this is because the forest basal area declines with increasing dry season length. This shows how environmental factors influence biomass accumulation through forest structure. For this reason, forest structure needs to be studied as possible predictors of biomass as well. These predictors are referred to as stand variables and include such variables as basal area, stem density, mean tree height, mean tree diameter, and mean wood specific gravity. Normally, these variables correlate strongly with AGB due to their direct relation to the allometric equations (Chave *et al.* 2005), although they do not always directly offer an ecological explanation for AGB. According to Baraloto *et al.* (2011) stand variables such as tree size, basal area and wood specific gravity are very appropriate for landscape-scale modelling of AGB, while soil and climate variables explain little variation. On a scale of the Amazon basin, Baker *et al.* (2004) too found that mean wood specific gravity is significantly related to the higher AGB values in the western Amazon. On the other hand, Stegen *et al.* (2009) could not detect such trend for various forest types across the Amazon basin, and in some

cases even found the opposite result. Furthermore, Hernández-Stefanoni *et al.* (2011) found that stand age was the most important biomass predictor. Hence, it seems that stand variables do play a role, yet overall scientific consensus on the exact contribution is still lacking.

Of the less pronounced environmental variables, soil type (chemical and physical) has been extensively studied, yet no clear result has been found. Overall, forest structure seems to be strongly related to soil type (Quesada *et al.* 2009b; Baraloto *et al.* 2011), while AGB on a landscape-scale is less effected (Clark & Clark 2000; Quesada *et al.* 2009b; Van der Laan *et al.* 2014). For the tropical dry forest of the Rio Grande - Valles Cruceños Natural Area of Integrated Management, this variable is less important as there are no large variations in main soil type for this area (FAO 2007).

One other factor on which no clear consensus exists is the effect of elevation gradients. Réjou-Méchain et al. (2014) showed a spatial autocorrelation in AGB for topographically heterogeneous sites at scales of more than 100 meters. They argued that topography should therefore be explicitly taken into account in sampling designs for ground-truthing of remote sensing data. De Castilho et al. (2006) also found a correlation between topography (both elevation and slope) and aboveground biomass. They argued that variations in topography create different sets of environmental conditions, which impact both forest structure and AGB. According to Marshall et al. (2012), this could be because trees are tallest at mid-elevation (1000-1250 m). These results are further emphasized by Ferry et al. (2010) who measured significant differences in total biomass between bottomlands and hilltops. They argued that this is mainly due to higher tree fall rates and waterlogged soils in bottomlands. For tropical forest on Borneo, Van der Laan et al. (2014) found that many variables were interrelated and that AGB could not be explained by a single environmental gradient, yet that elevation was the dominant variable among this set. On the other hand, Clark and Clark (2000) found that AGB is relatively insensitive to topography, but that it can have an effect on forest structure. For this reason, they recommend that landscape-scale studies of AGB account for this by employing stratified sampling designs. Girardin et al. (2014) explicitly studied the trends in biomass and forest structure across an elevation gradient in the Amazon to Andes transition zone of Ecuador, Peru and Bolivia. They found that tree height and AGB decreased with increasing elevation, while stem density increased. However, these studies were all done for tropical rain forest or cloud forest, and not much attention has yet been given to the effect of elevation on AGB in tropical dry forests

Overall, there seems to be scientific agreement that precipitation, and specifically the presence or absence and length of dry season has the strongest influence on aboveground biomass in tropical forests. However though, how strong this effect is, and how it relates to other factors such as mean annual temperature, stand variables or elevation, is unclear for tropical dry forests.

1.9 Spatial Variation in Plant Species Composition

One other aspect of tropical forests that is directly influenced by the dry season is the species distribution pattern. Engelbrecht *et al.* (2007) showed that plant distributions in tropical forest at local and regional scales are primarily influenced by plants' differential drought sensitivity. Tropical trees are directly distributed according to their sensitivity with respect to soil water availability. This suggest that plant species composition will probably be very distinct in tropical dry forests according to water availability. Furthermore, Aiba and Kitayama (1999) found that species diversity is also related to

elevation, decreasing with higher elevation. Both results suggest that topographically heterogeneous tropical dry forest such as the Río Grande - Valles Cruceños Natural Area of Integrated Management are likely to show a large diversity in species composition.

It was also found that tree species diversity correlates with tree height and estimated AGB for tropical forest on Borneo (Aiba & Kitayama 1999). However, on the Amazon to Andes elevation gradient, Girardin *et al.* (2014) found this did not directly correlate, with highest species richness at midelevation, while biomass showed a linear decrease with higher elevations. Furthermore, Fauset *et al.* (2015) recently found that for Amazon forests, the carbon storage function is skewed heavily towards relatively few "hyperdominant" species, with roughly 1 per cent of Amazonian tree species contributing to 50 per cent of carbon storage. Hyperdominant species are defined as those species that together account for more than 50 per cent of biomass. However, this "functional hyperdominance" has a strong geographical correlation, with certain hyperdominant species only present in certain Amazon regions. Compared with other parts of the Amazon, though, the southwestern Amazon, including the Bolivian tropical dry forest, shows the least hyperdominance (Fauset *et al.* 2015). Hence, it is thus not completely clear if and how species composition or the presence of hyperdominant trees in tropical dry forest is related to aboveground biomass storage.

1.10 Problem Definition and Research Aim

From the above, it follows that the problem at hand is threefold. Firstly, it has become clear that there is a large variety in the methodologies that are applied in order to estimate AGB. Most of these methodological tools are specifically designed for tropical rain forest. Thus, it is relevant to study the impact of various methodological choices that need to be made in order to estimate AGB in tropical dry forest. This is a methodological assessment.

Secondly, there is a necessity to establish accurate levels of aboveground biomass on a landscape-scale for the Río Grande - Valles Cruceños Natural Area of Integrated Management. Quantification of these biomass stocks is important for baselines studies of avoided deforestation. This will help to evaluate the REA conservation schemes as implemented by FNB, and its future application to REDD+. This problem is largely descriptive in nature, for which field-based data needs to be combined with remote sensing data. The desired end product consists of biomass distribution maps for the Río Grande - Valles Cruceños Natural Area of Integrated Management.

Thirdly, there is a necessity to better understand the predictive power of various stand and environmental factors on spatial variation in aboveground biomass in tropical dry forests, as well as on plant species composition and how this relates to AGB. This is partly a theoretical problem within ecology, partly a means to better address the previous problem.

From this, it follows that the aims of this research project are:

1. to study the effectiveness and accuracy of a regionally-applied field methodology for tropical dry forest;

- to map the regional variation in local AGB, forest structure and species composition in the Río Grande - Valles Cruceños Natural Area of Integrated Management, and provide a regional assessment of total AGB;
- 3. to analyse the predictive power of stand and environmental factors on regional spatial variation in AGB and species composition.

1.11 Research Question and Hypotheses

These three aims will be addressed though the following main research questions:

What is the influence of environmental factors on regional AGB estimates for avoided deforestation projects in the tropical dry forests of the Río Grande - Valles Cruceños Natural Area of Integrated Management, and how is this related to forest structure and species composition?

In order to answer this, the following sub questions will be assessed:

- 1. What are the effects of different methodological choices on estimated AGB values?
- 2. What is the regional variation in estimated AGB, forest structure and species composition across the Río Grande Valles Cruceños Natural Area of Integrated Management?
- 3. Which stand or environmental factors contribute most to regional spatial variation in AGB and species composition, and how can these be used for mapping regional AGB values?

Based on the literature, it is hypothesized that the choice of allometric equation has the largest impact on estimated AGB, followed by plot size.

Furthermore, it is hypothesized that AGB is correlated to stand variables and highest in regions with the shortest dry season and the highest annual precipitation. Precipitation is hypothesized to be a stronger predictor of AGB than elevation.

Species composition is hypothesized to differ significantly with precipitation conditions, and especially with the length of dry season.

2. Methodology

2.1 Study Area

Research was carried out in Bolivia, in the form of a research internship at Fundación Natura Bolivia. Field measurements were carried out on site in the Río Grande - Valles Cruceños Natural Area of Integrated Management, while remote sensing and GIS work was carried out at FNB's office in Santa Cruz de la Sierra.

The research area for this study was the Río Grande - Valles Cruceños Natural Area of Integrated Management (Área Natural de Manejo Integrado Río Gande - Valles Cruceños, or ANMI RG-VC) in Bolivia. The ANMI RG-VC is a departmental protected area in central Bolivia, created in 2007. Located about 100 km southwest of the city of Santa Cruz de la Sierra, it falls within the Department of Santa Cruz, bordering the Departments of Cochabamba and Chuquisaca (see Figure 1). Within its 734.000 ha area, the ANMI RG-VC comprises the municipalities of Cabezas, Gutiérrez, Samaipata, Vallegrande, Prostrervalle, Pucará, and Moro Moro. In total, it has a population of approximately 69.000 inhabitants (2001 census), of whom most are involved in agriculture and ranching. The ANMI RG-VC was formed with the aim of conserving its forests, reducing the impacts of flooding and droughts, and conserving biodiversity, while demonstrating the touristic potential of the area (Azurduy 2010).



Figure 1. *Location of the Río Grande - Valles Cruceños Natural Area of Integrated Management within Bolivia.* Source: Azurduy (2010).

The area is located in the centre of the Río Grande watershed, a hilly terrain in between the Andean altiplano and the Amazonian lowlands. Elevation ranges from 426 to 2982 meters, making it ideal for incorporating elevation as a predictive variable in this study. Annual precipitation is 878 mm on average, with a minimum of 281 and a maximum of 1764 mm. This characterizes most of the region's vegetation as tropical dry forest. However, as these figures show as well, precipitation differences are large, which results in a large ecological variation. Biogeographically, the area can be divided into 5 disitinct ecoregions: Tucuman-Bolivian forest (TB), Inter-Andean Dry forest (IAD), Chaco Serrano (CS), Gran Chaco (GC), and Yungas forest (Azurduy 2010). Here, an ecoregion is defined as a characteristic aggregation of natural communities that shares many taxa, ecological dynamics and environmental conditions (Ibisch & Mérida 2003). Figure 2 shows the distribution of these 5 ecoregions over the area. The primary focus of this research will be on Tucuman-Bolivian forest, Inter-Andean Dry forest, Inter-Andean Dry forest and Chaco Serrano, since these ecoregions encompass most of the area.

Tucuman-Bolivian forest is dense, evergreen to (semi-)deciduous, semi-humid, sub montane forest. This forest is generally dense and tall (20-30 m) and can be found in medium-to-high mountain ranges, with steep slopes (Carretero 2005). Evergreen forest is most likely found on lower slopes, or higher up in the form of tall Pine trees. It generally contains a wide variety of species. Floristically, it is very distinct from Chaco forest-types, but very close to Yungas forest. It generally experiences 3-5 months of dry season per year (Ibisch & Mérida 2003).

Yungas forest is humid, evergreen forest of small-to-large size (5-30 m high), in a mosaic of successional stages. It is very dense forest with a high level of diversity, and generally experiences little (0-2 months) dry season (Ibisch & Mérida 2003).

Inter-Andean Dry forest is seasonally dry, sparse, deciduous forest with high levels of endemism, especially various Cactaceae. Trees are medium-sized (max. 10-20 m). This type of vegetation can generally be found on plain to slightly hilly terrain, and experiences 6-8 months of dry season (Ibisch & Mérida 2003).

Chaco Serrano is a region characterized by medium-size mountain ranges (serrannías), with dry to semi-humid climate. Its forest is generally medium-sized dry deciduous forest (max. 25 m), and typically contains less Cactaceae than Inter-Andean Dry forest. Dry season generally lasts 6-7 months (Ibisch & Mérida 2003).

Finally, Gran Chaco is an almost plain region with dry climate. It contains low-growing dry deciduous forest with many Cactaceae, with high levels of endemism as well. Gran Chaco is floristically strongly related to Inter-Andean Dry forest, but generally harbours much lower plants (5-10 m). Dry season typically lasts 6-10 months (Ibisch & Mérida 2003).



Figure 2. The ANMI RG-VC area and its 5 ecoregions. Source: Ibisch & Mérida (2003).

Another way to classify the study area is according to ecosystem type. Navarro and Ferreira (2007) identified 19 distinct ecosystem types within the area. These classifications have a higher spatial resolution than the ecoregions by Ibisch and Mérida (2003), and are not based on biogeographic characteristics, but rather on specific vegetation type (Navarro & Ferreira 2007). The 8 main ecosystems in the ANMI RG-VC area are listed and described in Table 1. Together these 8 ecosystems comprise 94.4 per cent of the total ANMI RG-VC surface area. Figure 3 shows their distribution over the area.

Code (cf. Navarro	Ecosystem Description
& Ferreira 2007)	
CES409.213	Mountainous Tucuman-Bolivian seasonally-rained 'Matorral' shrub land
CES409.219	Mountainous Tucuman-Bolivian seasonally-rained 'Pajonal' scrubland
CES409.211	Inter-Andean - sub-Andean Tucuman-Bolivian xerophile forest
CES406.238	Sub-humid semi-deciduous forest of the Chiquitanía and Beni regions
CES409.206	Sub-humid Tucuman-Bolivian forest of the lower sub-Andean region
CES409.207	Sub-humid Tucuman-Bolivian forest of the upper sub-Andean region
CES409.197	Mountainous Tucuman-Bolivian forest with 'Pino de Monte' / Pine trees
CES409.205	Sub-Andean Tucuman-Bolivian forest in transition to Yungas

Table 1. List of	of 8 main	ecosystems	in the	ANMI	RG-VC stud	y area.
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Figure 3. *The ANMI RG-VC area and its 8 main ecosystems. For descriptions of CES-codes, see Table 1.* Source: Navarro & Ferreira (2007).

2.2 Remote Sensing Data

For remote sensing data, this research made use of RapidEye satellite imagery, provided by BlackBridge[®]. RapidEye provides high resolution Earth observation images in 5 main bands, namely red, green, blue, red edge, and near-infrared. It offers a specifically high spatial resolution of 5 m and is very well suited for identifying vegetation cover because of its use of a near-infrared band for detecting chlorophyll concentrations (Fox 2014). Remote sensing data was processed by Fundación Natura Bolivia in Exelis[®] ENVI software following a protocol as developed by Fox (2014).

To make use of RapidEye data, a land cover classification system is normally needed which provides a statistical basis for clustering pixels and classifying the image into different vegetation structural types (Quiñones *et al.* 2011). The main vegetation structural types that are generally used in these classifications are forested land, deforested land, other land covers with sparse vegetation, and shadow (Fox 2014). This classification can be done using supervised and unsupervised algorithms in ENVI software as detailed by Fox (2014). Subsequently, vegetation structural types can be matched with quantitative field data on AGB to create regional biomass maps with ArcGIS mapping software (Quiñones *et al.* 2011).

For this research, a classification was made into three vegetation structural types: 1) evergreen forest, 2) deciduous forest, and 3) all other vegetation types and non-forested areas (Uyuni 2015). In order to assess the accuracy of this classification, in situ verification of assigned vegetation structural types was

conducted by Fundación Natura Bolivia at various locations in the study area following a random sampling design as outlined by Fox (2014).

The resulting classified vegetation map of the ANMI RG-VC area was exported as raster data from ENVI into ArgGIS for further analysis. Using the intersect function, total surface area of both evergreen forest and deciduous forest was calculated for each of the five ecoregions, as well as for each of the 8 main ecosystem types in which field-based measurements were conducted. Based on this, total forest cover, as well as the relative distribution of evergreen and deciduous forests in each of the ecoregions and ecosystems was calculated. These relative distributions can be found in Table 2, while Figure 4 shows the spatial distribution of evergreen and deciduous forest in the entire ANMI RG-VC. As can be seen from the table, the 8 main ecosystems (covering 94.4 per cent of the total area) contain relatively more evergreen forest than is the actual case in the entire region.

Table 2. Total, evergreen and deciduous forest cover (in % of total area) for each of the 5 ecoregionsand 8 main ecosystems in the ANMI RG-VC.

Area	Surface area	Evergreen forest	Deciduous forest	Total forest
		cover	cover	cover
	(% of total	(% of total	(% of total	(% of total
	ANMI RG-VC)	ANMI RG-VC)	ANMI RG-VC)	ANMI RG-VC)
Tucuman-Bolivian	37.2	22.1	7.1	29.1
Inter-Andean Dry	12.3	3.0	5.6	8.6
Chaco Serrano	37.9	18.0	14.5	32.6
Gran Chaco	11.7	3.9	6.4	10.3
Yungas	1.0	0.5	0.1	0.6
Total ANMI RG-VC	100	47.5	33.8	81.3
CES409.213	3.3	0.9	1.6	2.5
CES409.219	4.8	1.2	0.9	2.1
CES409.211	26.8	10.8	12.6	23.4
CES406.238	12.8	8.0	3.2	11.2
CES409.206	8.7	6.9	0.9	7.8
CES409.207	20.7	13.4	4.7	18.1
CES409.197	10.2	6.9	0.7	7.6
CES409.205	7.2	4.4	1.0	5.4
Total 8 Ecosystems	94.4	52.5	25.6	78.1



Figure 4. Spatial distribution of evergreen (green) and deciduous (red) forest in the ANMI RG-VC, based on RapidEye remote sensing data. White spaces are non-forested areas.

2.3 Field-Based Measurements

In order to quantify AGB estimates and answer the various research questions, field-based measurements were carried out in the form of temporary and permanent forest plots. For this, a stratified, factorial sampling design was used. A stratified design was chosen because field measurements were distributed over 4 of the main forest type classes in the research area: both evergreen and deciduous forests within the Tucuman-Bolivian forest ecoregion, as well as deciduous forest within both the Inter-Andean Dry forest ecoregion and the Chaco Serrano ecoregion. Here, a forest type class is defined as a combination of an ecoregion and one of the vegetation structural types: evergreen or deciduous forest. Evergreen forests within the Inter-Andean dry forest was not included as a separate class, because of its relatively small contribution to total forest cover in the ANMI RG-VC, while evergreen Chaco Serrano was excluded because Chaco Serrano is generally considered to be deciduous forest (Ibisch & Mérida 2003). The Yungas ecoregion was left out completely because of its relatively minor presence in the ANMI RG-VC. Furthermore, the Gran Chaco ecoregion was also left out of the field measurements because Gran Chaco data have already been collected before for Fundación Natura Bolivia by Arnstein (2014).

A factorial design was used because forest type was not the only factor to be taken into account in the sampling design. In order to be able to properly assess the effect of elevation as a possible environmental predictor of forest AGB, elevation was used as a second, stratified factor in the design.

However, this could only be done for evergreen Tucuman-Bolivian forest and deciduous Inter-Andean Dry forest, as the other two forest type classes did not show sufficient elevation gradients in their geographical distribution. Thus, 5 elevation strata were identified, ranging from 500-1000 meter above sea level to 2500-3000 m. In total, this meant that 8 specific combinations of elevation and forest type class were identified, as shown in Table 3.

Based on these combinations, 8 different local communities or villages in the ANMI RG-VC region were identified for field-based measurements. These were the following communities: Chiriguanañan, Algodonales, Molleaguada, Salsipuedes Grande, Laja Toco, Pampa Negra, Bicoquin, and Tocopampa.

Table 3: Overview of the stratified, factorial sampling design. The 8 sites are given in **bold italics.** Each CES-code represents a single forest plot. For an explanation of CES-codes, see Table 1. NA: Not applicable; MV: Missing value.

Elevation	Evergreen	Deciduous	Deciduous	Deciduous	
	Tucuman-Bolivian	Tucuman-Bolivian	Inter-Andean Dry	Chaco Serrano	
	forest	forest	forest	forest	
500-1000 m	NA	NA	NA	Laja Toco:	
				CES406,238	
				CES406,238	
				CES406,238	
1000-1500 m	Algodonales:	Tocopampa:	Pampa Negra:	NA	
	CES409,206	CES409,207	CES409,211		
	CES409,206	CES409,207	CES409,211		
	CES409,206	CES409,207	CES409,211		
1500-2000 m	Bicoquin:	NA	Molleaguada:	NA	
	CES409,205		CES409,211		
	CES409,207		CES409,211		
	MV		-		
2000-2500 m	Chiriguanañan:	NA	CES409,213	NA	
	CES409,197				
	CES409,197				
	CES409,197				
2500-3000 m	Salsipuedes Grande:	NA	NA	NA	
	CES409,219				
	MV				
	MV				

At each of the 8 chosen sites, 3 forest plots were installed in locations representative of the factorial combination. This meant that ideally, the sampling design encompassed 24 samples. However, 1 plot in the community of Bicoquin could not be installed, due to logistical difficulties in the field because of heavy rainfall. Furthermore, the actual forest cover in Salsipuedes Grande was very small and located on steep terrain, which meant that only 1 plot could be installed at this site. Also, due to problems with contacting forest owners in the field, one of the plots in Molleaguada could not be located in the correct elevation stratum of 1500-2000 m, and was thus located in the stratum from 2000-2500m.

These deviation from the original design are indicated in *red* in Table 3. Forest plot locations could also be classified according to ecosystem type as identified by Navarro and Ferreira (2007). These ecosystem types are indicated by the respective CES-codes in the table. In total, only 21 plots out of the ideally desired 24 plots were installed.

Contrary to what is often the case in this type of stratified sampling designs (Lepš & Šmilauer 2003), these 8 sites or 21 plot locations were not chosen at random. Instead, locations were identified based on the following criteria: accessibility by road, presence of REA conservation areas, absence of steep elevation gradient, and general representativeness for the chosen forest type. Locations needed to be accessible by 4x4 vehicle, in order to save time and easily transport research equipment and collected field material. Furthermore, plots needed to be installed within current REA sites, so that the plots are likely to not be disturbed or degenerated and future remeasurements will be possible. Also, ideally, plots were installed on flat surface. However, in practice, this proved to be difficult and the presence of a slope was unavoidable in certain cases. Lastly, within the constraints of the previous criteria, a plot location needed to be reasonable representative for the area. Figure 5 shows the chosen locations within the ANMI RG-VC region.



Figure 5. Location of the 8 sites within the ANMI RG-VC. Also shown are topography and the main roads within the area.

Forest inventory measurements were carried out at each plot following a nested sampling protocol. This protocol was specifically designed for this study in the ANMI RG-VC in order to allow for a maximum study of both regional variation as well as local variation in aboveground biomass, within

the temporal and financial constraints of this research. It is an adaptation of the commonly applied 1 ha RAINFOR protocol (Phillips *et al.* 2015), the IBIF protocol by Villegas *et al.* (2009) and the BOLFOR and PROMABOSQUE protocol by Contreras *et al.* (1999). Adjustments were based on first field experiences with a 1 ha plot in the community of Tocopampa, and made after close consultation with expert botanists at the Noel Kempff Museum of Natural History in Santa Cruz de la Sierra.

Each of the 3 plots installed at each site measured 20x50 m or 0.1 ha, which makes a total of 0.3 ha per site or factorial combination. This follows the recommendations by Baraloto *et al.* (2013), who argue for using a stratified sampling strategy consisting of several smaller (<1 ha) plots with at least 3 sample plots for each vegetation structural type. However, both Baraloto *et al.* (2013) and Chave *et al.* (2004) recommend using a minimum plot size of 0.25 ha. Due to temporal and financial constraints, it was nonetheless chosen to install three 0.1 ha plots, rather than one 0.25 ha plot. Although this approach is slightly more labour-intensive and more susceptible to type 3 estimation errors, it allows for statistical comparison of the local variation within the 3 plots with the between-group variation of other sites. This would not have been possible with only one 0.25 ha plot. Furthermore, with 3 smaller plots, one is likely to obtain a better representation of the forest landscape than with just 1 plot, reducing a possible type 4 error (Chave *et al.* 2004). Hence, in the case of a topographically and ecologically heterogeneous landscape such as in the ANMI RG-VC, this approach is preferable, as local variability in forest type and elevation is large.

Nonetheless, as Jayakumar *et al.* (2011) point out, the 20x50 m or 0.1 ha plot it is a plot technique that has been applied very little. Only Killeen *et al.* (1998) used it for tropical semi-deciduous forest in the Chiquitanía region of Santa Cruz, while Luza (2015) applied it to mountainous Andean forest in Peru. This seems to indicate that this method might indeed be more suitable for application to topographically heterogeneous landscapes and/or tropical dry forest, than homogeneous tropical rain forest in the Central Amazon.

A nested sampling design was used, which means that there were 3 measurement levels. Firstly, within the 20x50 m plot, all trees with a diameter at breast height (DBH \approx 1.30 m) larger than 5 cm, were measured. Contrary to common practice, the 5 cm DBH limit was used, instead of 10 cm, because most of the trees in Inter-Andean Dry forests are likely to fall within the 5-10 cm DBH class. In order to be able to properly compare this type of vegetation with other areas, a minimal DBH limit of 5 cm was set for all sites. For each tree that fell within the DBH \geq 5 cm class, its DBH, estimated tree height and common species name were recorded. Tree height of each tree was estimated by eye and based on consensus among the field workers. If the common species name could not be identified, a leaf sample was taken for further botanical identification at the Noel Kempff Museum of Natural History. All lianas with a diameter \geq 5 cm were measured as well, and marked as *Liana* in observations. All fallen or standing dead trees with a diameter \geq 5 cm were measured too, and marked as *Necromass* in the observations.

Proximity between the three forest plots in each of the 8 sites generally varied from 200 to 500 meters, depending on local terrain conditions and the location criteria as specified. The exact location of the 4 corners of each plot was recorded using standard GPS devices. Out of the 3 plots, 1 plot was a permanent forest plot, while the other 2 were temporary. In the permanent forest plots, all trees with DBH \geq 10 cm were marked with an individual number imprinted on an aluminium plaque that was

nailed in the bark at 20 cm above breast height (\approx 1.50 m). Also, X,Y-location within the forest plot was noted for each of these trees. This data was recorded in order to study forest dynamics using a remeasurement 5 years from now. This was not done for trees in the temporary plots.

The second and third level of the sampling design applied only to the 1 permanent forest plot at each site. At each corner of this main plot, a subplot of 5x5 m was installed, in which all small living trees or bushes with DBH between 2.5 and 5 cm were measured. For each of these trees, common species name, DBH, and tree height were noted.

The third level then consisted of installing 1x1 m quadrants at each corner of the permanent plots, in order to collect all plant litter, grasses, and herbaceous vegetation. A diagram of the nested sampling design can be found in Figure 6.



Figure 6. Diagram of the nested sampling design of 1 permanent forest plot and 2 temporary forest plots.

However, in the Tocopampa community, the sampling design looked somewhat different. This site was first sampled for a different study of Fundación Natura Bolivia, in the form of a 1 ha plot. Yet its location was suitable as deciduous Tucuman-Bolivian forest in the 1000-1500 m elevation range. For this reason, it was decided to incorporate the Tocopampa data in this study as well. For this, three subplots of 20x50 m were identified within the original 1 ha plot. Based on X,Y-data of all the trees in the original plot, those trees that fell within one of these 20x50 m subplots were used for a separate calculation of AGB within this 0.1 ha subplot only. However, contrary to the other plots, only trees with DBH \geq 10 cm

were measured in the original 1 ha plot. For this reason, data on the 2.5-10 cm DBH class was taken from a 2x100 m transact within the same 1 ha plot. This approach had the additional advantage that it would allow for a comparison between the 3x0.1h plot approach and the 1 ha plot approach. This would therefore help address the methodological research question of this study.

2.4 Calculations of Aboveground Biomass

Seven different allometric equations were used to calculate aboveground biomass. These are listed in Table 4. These models were based on 4 original models by Brown *et al.* (1989), Brown (1997), Chave *et al.* (2005) and Chave *et al.* (2014). Since no region-specific allometric equations exist, it was decided to use the dry forest version of each allometric model. Only Chave *et al.* (2014) represents a pantropical model that is not specifically designed for tropical dry forest. The Brown models do not include wood density (ρ) while most of the Chave models do. Furthermore, of the Chave *et al.* (2005) model, three versions exist: one without tree height, one with three height estimated by eye, and one with a calculated tree height according to the regional-specific Weibull-H model by Feldpausch *et al.* (2012). This is a regional allometric model linking tree height H to DBH values (see Table 5).

Equation #	Author (forest type)	Year	Density (ρ) included?	Height (H) included?	Type of tree height estimation	Allometric equation
1	Brown <i>et</i> <i>al.</i> (dry)	1989	No	No	-	AGB (kg) = 34,4703 - 8,0671 * DBH + 0,6589 *(DBH^2)
2	Brown (dry)	1997	No	No	-	AGB (kg) = EXP(-1,996 + 2,32 * LN(DBH))
3	Chave <i>et</i> <i>al.</i> (dry)	2005	Yes	No	-	AGB (kg) = ρ * EXP(-0,667 + 1,784 * LN(DBH) + 0,207 * (LN(DBH))^2 - 0,0281 * (LN(DBH))^3)
4	Chave <i>et</i> <i>al.</i> (dry)	2005	Yes	Yes	Field estimate by eye	AGB (kg) = 0,112 * (ρ * DBH^2 * H)^0,916
5	Chave <i>et</i> <i>al.</i> (pan- tropical)	2014	Yes	Yes	Field estimate by eye	AGB (kg) = 0,0673 * (ρ * DBH^2 * H)^0,976
6	Chave <i>et</i> <i>al.</i> (dry)	2005	Yes	Yes	Allometric model (Feldpausch <i>et al.</i> 2012)	AGB (kg) = 0,112 * (ρ * DBH^2 * H)^0,916
7	Chave <i>et</i> <i>al.</i> (pan- tropical)	2014	Yes	Yes	Allometric model (Feldpausch <i>et al.</i> 2012)	AGB (kg) = 0,0673 * (ρ * DBH^2 * H)^0,976

Table 4. Overview of the 7 allometric equations that were applied in this study.

Wood density of each species was taken from the Global Wood Density database (Chave *et al.* 2009; Zanne *et al.* 2009). When the species name of an individual plant was unknown, the average density of all South American (tropical and extra-tropical) species within the family or genus was used. If the genus or family level was unknown as well, the average of all the living species in the individual's forest plot was used. For cactuses, only the wood density of *Neoraimondia herzogiana* was known, which was then taken as an average for all Cactaceae. In the case of a liana, no density or height was used, since liana species names could often not be identified and measuring the total length of liana is extremely difficult in a dense forest. Instead, following Addo-Fordjour and Rahmad (2013), a specific allometric equation for lianas was used, which only includes DBH (Equation 9, Table 5). For dead woody biomass, no generally agreed-upon equations exist (Linneo & Lazarte 2012). Yet it is necessary to account for decomposition processes in coarse woody debris (Baker *et al.* 2007). For this reason, following Arnstein (2014), it was decided to take 70 per cent of the estimated biomass in order to account for biomass loss due to decomposition (Equation 10, Table 5). Since the species name of dead trees could not be identified in most cases, the average density of all the known living trees in that forest plot was then used to calculate necromass.

Equation #	Author	Application	Equation
8	Feldpausch <i>et al.</i> (2012)	Western Amazon Weibull-H allometric model for estimating tree height	H (m) = 227,35 * (1 - EXP(-0,0139 * (DBH^0,555)))
9	Addo-Fordjour & Rahmad (2013)	Lianas	AGB (kg) = 10^(0,49 + 1,09 * (LOG10(DBH)))
10	Cf. Arnstein (2014)	Necromass	ABG (kg) = 'Result Eq. 1- 7' * 0,7

Table 5. Overview of 3 additional equations used in this study.

The collected plant litter, grasses and herbaceous vegetation of the 1x1 m subplots was weighed with a normal balance. Subsequently, humidity and dry weight was calculated after drying thoroughly mixed samples of ca. 100 g for several days at 65 °C in a stove. The average dry weight (in g/m²) of the 4 subplots was then used to calculate the biomass value of plant litter, grasses and herbaceous vegetation (in tonnes/ha) for each site. Similarly, the measurements of small living trees in the 5x5 m subplot of the permanent forest plot were used for the 2 temporary plots at that same site as well.

With this method, results were obtained for 5 biomass compartments: big living trees (DBH \geq 5 cm), small living trees (DBH 2.5-5 cm), dead trees (DBH \geq 2.5 cm), lianas (DBH \geq 2.5 cm), and plant litter, grasses and herbaceous vegetation. For all of these, results were delivered as AGB values in tonnes/ha only, and not converted to carbon values. This allowed for more straightforward comparison with other studies. Total aboveground biomass was calculated by summing the results of the 5 compartments. Hence, total AGB is defined here as including both living and dead biomass.

2.5 Collection of Environmental Data

Environmental data for each of the 21 plot locations was obtained from various sources. Elevation levels were taken from a 30 m resolution ASTER global digital elevation model (DEM) (JSS 2011). Furthermore, various bioclimatic variables were collected as possible predictor variables. These included: mean annual temperature (°C), mean annual precipitation (mm), precipitation seasonality (coefficient of variation), average monthly precipitation (mm), and average precipitation of the driest quarter (mm). This data was taken from the WoldClim global climate model (Hijmans *et al.* 2015).

Additionally, the long-term climatic water deficit (CWD) and the environmental stress factor (E) were taken from Chave *et al.* (2014) as possibly related climatic variables. The long-term climatic water deficit is the water lost by the environment during dry months (when evapotranspiration exceeds rainfall). In the case of this study, it assumes 1 dry season per year. It is measured as the difference (in mm/yr) between rainfall and evapotranspiration during dry months only. Hence, it is by definition negative (Chave *et al.* 2014). The environmental stress factor E is an environmental variable that was found to be an important covariable of the diameter-height relationship of tropical trees. It is calculated based on temperature seasonality, CWD, and precipitation seasonality (Chave *et al.* 2014).

As an additional measure for dry season impact, NDVI differences between August 2005 and January 2005, and between August 2010 and December 2010, were calculated based on the RapidEye remote sensing data from Uyuni (2015). The difference in NDVI values (i.e. presence of vegetation) between dry and wet season can be seen as an additional measure for the dry season impact.

2.6 Data Analysis

In order to answer the research question, various forms of data analysis and statistical tests were conducted. Statistical tests were all carried out using IMB SPSS Statistics 23 software, unless specified otherwise.

The first sub question read: *What are the effects of different methodological choices on estimated AGB values?* Unfortunately, no complete sensitivity analysis could be conducted to answer this, as the dataset was too small for this purpose. Instead, the 3 most important methodological choices were analysed independently, and error size was calculated for each of them as a percentage change.

The first choice to study was the effect of the choice of allometric equation on AGB estimates. This was analysed by comparing the mean total AGB estimates of each of the 7 equations over all 21 forest plots. Shapiro-Wilk's tests were performed in order to check for normal distributions. In order to analyse the variation among the means, a repeated-measures ANOVA (Analysis of Variance) was carried out. Repeated-measures ANOVA was used in this case, because all 7 equations are consistently applied to the same dataset of 21 forest plots, which thus resembles repeated measures of the same samples. Mauchly's test of sphericity was also performed, because sphericity of data is an important assumption of a repeated-measures ANOVA.

The second choice to study was the choice between 1 ha or 0.1 ha plot size. The effect of this was studied by analysing the data from Tocopampa, in the Tucuman-Bolivian forest ecoregion. In Tocopampa, data from both a 1 ha forest plot and three 0.1 ha forest plots were obtained from the

same forest area and could thus be compared. For this, the mean AGB value of the 3 smaller plots was compared with the single AGB value of the larger 1 ha plot. Statistically comparing these differences was not possible though, since the 1 ha plot group has n=1. However, the 0.1 ha mean and the original 1 ha value could also be seen as the outcomes of 2 different treatment groups, each with 7 samples (the 7 allometric equations). Since these groups were related (the same forest area), this could statistically be tested using a paired-samples t-test.

The last methodological choice to be analysed was the choice for a lower DBH measurement limit at 5 or 10 cm. For this, Tucuman-Bolivian and Chaco Serrano forest plots that had X,Y-coordinates of trees were used to calculate a new AGB value based on solely those trees with DBH \ge 10 cm. This was done for the plots in Algodonales, Chiriguanañan, Laja Toco and Bicoquin. Salsipuedes Grande lacked X,Y-coordinate data, and Tocopampa lacked DBH 5-10 cm data which is needed for the comparison. IAD forest plots were not included since the majority of trees in these plots tends to be in the 5-10 cm DBH class. In the chosen plots, those trees with a DBH between 5 and 10 cm located within the 5x5 m subplots were added to the AGB of small living trees with DBH 2.5-5 cm in order to form a small living trees in these plots. This was done for Eq. 2 and 6 only. To analyse the differences, a non-parametric test for related samples, the related-samples Wilcoxon Signed Rank test, was used.

The second sub question read: *What is the regional variation in estimated AGB, forest structure and species composition across the Río Grande - Valles Cruceños Natural Area of Integrated Management?* However, there are various ways to analyse the regional variation in AGB, depending on the spatial level. One can analyse this at ecoregion level, forest type class level, ecosystem level, or site level.

Firstly, the differences in mean total AGB between the 8 sites were analysed. Mean total AGB was calculated as the average of total AGB of the 3 forest plots that were located at each site. Exceptions were the community of Bicoquin (TB forest), were a mean AGB was calculated based on 2 forest plots only, and the community of Salsipuedes Grande (also TB forest), which only had 1 plot. Analysis was carried out on the results from equation 2 and 6 only, because these were the only results that showed a normal distribution. A one-way ANOVA was conducted with these results, with a site dummy variable as independent variable. Levene's tests for homogeneity of variances were performed, as well as Tukey's post hoc tests. Since Salsipuedes Grande only had 1 forest plot, these tests were carried out without including Salsipuedes Grande. To complement this, a non-parametric Kruskal-Wallis test that included the data from Salsipuedes Grande was also conducted. In order to further quantify the difference in local and regional variation, the regional variation (n=21) was compared with the average local variation (n=8) of each site (n=3), for all the 7 cases of different allometric equations. This was done using a paired-samples t-test.

Next, the differences in total AGB between the 3 main ecoregions, as well as between the 4 forest type classes were assessed using a one-way ANOVA. Again, this was done for Eq. 2 and 6 only. Furthermore, to test for their combined effects, a factorial ANOVA was conducted with these two main factors: forest type class and elevation.

Thirdly, the differences in total AGB between the various ecosystems were analysed. For this, only those ecosystems that appear multiple times within the sampling population were taken into account. Thus, this involved: Inter-Andean - sub-Andean Tucuman-Bolivian xerophile forest (CES409.211), Sub-

humid semi-deciduous forest of the Chiquitanía and Beni regions (CES406.238), Sub-humid Tucuman-Bolivian forest of the lower sub-Andean region (CES409.206), Sub-humid Tucuman-Bolivian forest of the upper sub-Andean region (CES409.207), and Mountainous Tucuman-Bolivian forest with 'Pino de Monte' / Pine trees (CES409.197). Again, a one-way ANOVA was carried out on the results of Eq. 2 and 6, as well as Levene's tests for homogeneity of variances and Tukey's post hoc tests. This analysis was also done with elevation as a covariate. Besides, a non-parametric Kruskal-Wallis test was performed on the complete dataset with all 8 ecosystem types.

Next to analysing these differences, the relative contribution of the 5 main biomass compartments to total AGB was also studied for each of the 8 sites. A one-way ANOVA was conducted to analyse the difference in the relative contribution of big living trees to total AGB between these sites.

Differences in forest structure between the 8 sites were studied by conducting one-way ANOVA tests on various forest structure variables: basal area (the amount of m² of trees/ha), stem density (the total amount of trees/ha), mean wood density (in g/cm³, for DBH \geq 5 cm), biomass in various DBH classes (5-10, 10-20, 20-30 cm, etc.), as well as stem density in these DBH classes. In most of these cases, the assumption of homoscedasticity was violated. For this reason, it was decided to conduct nonparametric Kruskal-Wallis tests in all cases. Since a different sampling methodology was applied in Tocopampa (with DBH \geq 10 cm), forest structure data from that community was not comparable with the other data and consequently left out in all these tests.

Species composition was analysed as well. The complete species dataset of all 21 plots was analysed for abundance of plant families. Also, it was analysed for IVI-B values per plant family. IVI-B is the biomass-adjusted Importance Value Index. The Importance Value Index gives a value to the relative importance of each species in a plant community by calculating the average of its abundance (relative stem density), dominance (relative basal area), and frequency. However, frequency is not applicable to this study, since this refers to the frequency of species presence in various subplots within a larger 1 ha plot (Matteucci & Colma 1982). This was not measured for this study. Another option, though, is to include the relative contribution of each species to total biomass as a measure of its importance. This then becomes the biomass-adjusted Importance Value Index. It gives a better index for the relative importance of species than just abundance and dominance alone, especially from a carbon storage perspective (Linneo 2014). For this study, without including frequency, IVI-B was calculated as follows:

$$IVI - B(i) = (ARi + DRi + BRi)/3$$
 (Eq. 11)

where ARi = the relative abundance of species i, DRi = the relative dominance of species i, and BRi = the relative total biomass of species i. With this data, the most important species were identified for each site. Furthermore, hyperdominance was analysed by identifying for each site those species that together contribute more than 50 per cent of total biomass. The hyperdominant character of the vegetation was then analysed by calculating the relative number of hyperdominant species for each of these locations.

The third sub question read: *Which stand or environmental factors contribute most to regional spatial variation in AGB and species composition, and how can these be used for mapping regional AGB values?* In order to answer this sub question, a multivariate analysis was carried out in CANOCO 5. CANOCO 5

is a multivariate statistics program specifically designed for the field of ecology. Multivariate statistics is very useful for finding the main factors or gradients that determine changes in large, species-rich ecological data (Lepš & Šmilauer 2003). For this multivariate analysis, the biomass-adjusted Importance Value Index of each species was used as dependent data, while stand and environmental variables were used as independent variables composed of compositional data for all 21 plots.

Via unconstrained ordination in the form of a detrended correspondence analysis (DCA), the total variation in the plant species dataset was first analysed. This analysis allows for a general comparison of the species composition and diversity between different sites. It is called an unconstrained ordination, because the analysis of variation is not yet constrained by predictor variables.

A constrained analysis was also carried out on the same dataset in the form of a canonical correlation analysis (CCA). Contrary to DCA, this allows for the inclusion of stand and environmental variables as possible predictors of the variation in species composition. In this way, it is possible to identify principal axes of variation in the dataset and the environmental predictors that are associated to these axes. For this analysis, 3 stand variables were used, namely total stem density, basal area, and mean wood density (MWD). As environmental variables, the following 12 variables were used: elevation (DEM), mean annual temperature (AnTemp), mean annual precipitation (AnPrecip), precipitation seasonality (PS), average monthly precipitation in August (Prec8), average precipitation of the driest quarter (PDQ), climatic water deficit (CWD), environmental stress factor (E), the difference in average monthly precipitation between January and August and between December and August (Diff 1-8 and Diff 12-8), and the difference in NDVI values between January 2005 and August 2005 and between August 2010 and December 2010 (NDVI Dif 1 and NDVI Dif 2). Average monthly precipitation in August was chosen as a separate variable, because August is generally the driest month in the ANMI RG-VC, and this might thus be an important predictor of variation.

Multivariable analysis was also carried out on total AGB results, the difference with multivariate analysis being that in this case there is only 1 dependent variable (namely, AGB). This was done via a generalized linear model analysis of the variation in total AGB over all 21 plots, with the 12 environmental factors as predictor variables. A redundancy analysis (RDA) of the variation in AGB in relation to stand variables was also performed. However, this could only be done for the 18 plots without Tocopampa, as the stand data from this community is not comparable with the other data (DBH \geq 10 cm).

Next, a step-wise linear regression analysis was performed, again in SPSS, in order to compose more simple regression equations for AGB modelling. With the results of Eq.2, this was done for the entire dataset, hence covering all 3 main ecoregions. For the Tucuman-Bolivian forest ecoregion only, this was also done with both the results of Eq.2 and Eq.6. With the resulting regression equations, AGB values could spatially be modelled in the Tucuman-Bolivian forest ecoregion. With the remote sensing data on vegetation cover, this made it possible to calculate total AGB in the Tucuman-Bolivian forest ecoregion, both for Eq.2 and Eq.6. For the other ecoregions, an average AGB value was used to calculate region-wide biomass. An estimate for the entire ANMI RG-VC was then obtained by summing all these values.

3. Results

3.1 Effects of Methodological Choices

In this first part of the analysis, we will look at the variation that is caused by various methodological choices. This will address the first sub question of this research: *What are the effects of different methodological choices on estimated AGB values?*

3.1.1 Effect of Allometric Equation

Various methodological choices had to be made in order to collect and analyse the data, which each influence the final outcome. The first of these choices to look into is the effect of the 7 allometric equations that were used to calculate estimated AGB values.

Means and standard deviations of total AGB for each of the 7 equations are shown in Figure 7. As can be seen, standard deviations tend to increase for more recent equations. Especially equations 3 to 7 show more variation, which is probably because these equations contain 1 more variable, namely density (ρ). An overview of the AGB outcomes per equation for each single forest plot can be found in Figure 8. This shows that the variation in AGB tends to increase for plots with higher AGB values.



Figure 7. Mean total AGB over all 21 plots for allometric equations 1-7.



Figure 8. Total AGB results of all 21 plots for each of the 7 allometric equations.

The Shapiro-Wilk's normality test showed that only 2 of the 7 formulas are normally distributed across the 21 plots. These are equation 2 (Brown 1997; p=0,054) and equation 6 (Chave *et al.* 2005, with tree height following Feldpausch *et al.* 2012; p=0,063). This indicates, firstly, that these formulas are most suitable to be used in any further analysis. For this reason, in further analyses and calculations, only these 2 equations are used, unless specified otherwise.

According to Mauchly's test, the assumption of sphericity was violated (χ^2 =294.462; p=0.000). For this reason, a Greenhouse-Geisser correction was applied. With this correction, the repeated-measures ANOVA showed that there are statistically significant differences between the 7 formulas (F=33.518, p<0.0005). Post-hoc tests using a Bonferroni correction revealed that equations 3, 6 and 7 produce significantly higher results than Eq. 1, 2, 4 and 5. Furthermore, it was shown that there are minor, yet statistically significant differences between Eq. 1, 2 and 3. Differences between equations 3, 6 and 7 are not significant.

The differences between Eq. 6 and 7 and 4 and 5 are bigger than the differences between Eq. 3 and 1, 2, 4 or 5. The former is a difference of 40-45 per cent, and the latter 20-35 per cent. This shows that the difference between estimating tree height by eye (as in Eq. 4 and 5) or calculating it using the Feldpausch *et al.* (2012) allometric equation (as in Eq. 6 or 7) has a larger effect on the final results than the choice of the original formula (Eq. 1-5).

3.1.2 Effect of Plot Size

Another methodological choice that could have a strong impact on final results was the choice of plot size: in this case, 0.1 ha plots compared with regular 1 ha plots. Figure 9 shows the results of the mean AGB value of the 3 smaller plots as well as the single larger plot in Tocopampa for all 7 allometric equations. Error bars show standard deviations for the mean of the smaller plots. Since there is only 1


sample for the 1 ha plot group, these values lack error bars. As one can see, in all 7 cases, the 1 ha plot value falls within the error margin of the 0.1 ha plot mean. This suggests there are no big differences.

Figure 9. AGB results of the 0.1 ha plots and 1 ha plot in Tocopampa, for all 7 equations.

The paired-samples t-test (Figure 10), though, shows that there are significantly lower result for the 0.1 ha plots, averaged over all the 7 formulas (t=7.086; p=0.000). However, it must be mentioned that this test is not entirely valid in this case, since the two paired groups are not exactly the same. One group is part of the other. For this reason, we cannot draw firm conclusions from this test. These results only indicate that the 0.1 ha plot might slightly underestimate AGB, by ca. 10 per cent, for Tucuman-Bolivian forest in Tocopampa.



Figure 10. *Diagram of the paired-samples t-test for the 0.1 ha and 1 ha forest plots, averaged over all allometric equations.*

3.1.3 Effect of Lower DBH Limit

One other factor that was studied is the choice to include all trees with a DBH \geq 5 cm in our dataset. This differs from the starting point of DBH \geq 10 cm, which is more common in other forest plots. Thus, it is relevant to compare the effect of this choice on final total AGB outcomes. For both allometric equations 2 and 6, this produces the following graph, as in Figure 11.



Figure 11. AGB estimates with $DBH \ge 5$ and $DBH \ge 10$ cm, for Eq. 2 and 6.

The values for these 11 plots were not normally distributed (Shapiro-Wilk's test: p=0.007 and p=0.032 for the two equations). Thus, a non-parametric test for relates samples was used: the related-samples Wilcoxon Signed Rank test. No differences were found with this test (p=0.709). Thus, it can be concluded that the DBH limit does not have an effect on estimated AGB for Tucuman-Bolivian or Chaco Serrano forest.

3.2 Analysis of Spatial Variation

We will now look into the second sub question of this research, which ran: *What is the regional variation in estimated AGB, forest structure and species composition across the ANMI RG-VC region?* We will firstly look at the regional variation in aboveground biomass, then at the biomass distribution across the various forest compartments, followed by the variation in forest structure and species composition.

3.2.1 Spatial Variation in Aboveground Biomass

There are various ways to study the variation in AGB. Firstly, we will look at the differences in AGB at site level. These results can be seen in Figure 12, and the exact figures can be found in the appendix. It was found that aboveground biomass values in the research area range from 77 tonnes/ha in Molleaguada to 350 tonnes/ha in Bicoquin (in the case of Eq.2), or from 106 tonnes/ha in Pampa Negra to 512 tonnes/ha in Bicoquin (in the case of Eq.6).

Levene's tests for homogeneity of variances were found to be insignificant (p=0.064 and p=0.069 respectively), which meant that the assumption of homoscedasticity was met. For both allometric

equations, the one-way ANOVA showed significant between-groups differences (F=26.543 and p=0.000; F=14.613 and p=0.000, respectively). Tukey's post-hoc tests revealed that Bicoquin has significantly larger AGB values than all the other sites. Also, the two Inter-Andean Dry forest sites (Pampa Negra and Molleaguada) are significantly different from the Chaco Serrano site (Laja Toco), which generally has a larger AGB than most of the Tucuman-Bolivian forest sites. Only in the case of Eq. 2 are the Inter-Andean Dry forest sites also significantly different from other Tucuman-Bolivian forest locations, namely Chiriguanañan and Tocopampa. When we include Salsipuedes Grande in the analysis, by running a non-parametric Kruskal-Wallis test, this also reveals significant differences between the groups (p=0.016 and p=0.030, respectively).



Figure 12. Mean total AGB (n=3) per site for equation 2 and 6.

The largest local variation (between the 3 local plots at each site) can be found in Bicoquin and Laja Toco. Figure 12 also shows the regional mean, averaged over all 21 plots, and its variation. On average (n=8), the local variation between the 3 plots at each site has a standard deviation of 22.7 or 47.4 (Eq. 2 and 6, respectively). In the case of regional variation (n=21), however, the standard deviation is 80.3 or 121.1 for Eq. 2 and 6. Although the regional mean is even a bit lower than the mean of the 8 local sites averages, the regional standard deviation is 3 to 4 times as high as the average local standard deviation (Figure 13).

This can be quantified with a paired-samples t-test. A paired-samples t-test with all 7 allometric equations shows that the regional standard deviation is substantially higher (x2.9) than the local standard deviation, and significantly different (t=19.604; p=0.000).



Figure 12. Comparison of the local and regional mean AGB and standard deviations for Eq. 2 and 6.

Next to studying the differences at site level, we can also look at the differences averaged over the various ecoregions and forest type classes. For both Eq. 2 and 6, a one-way ANOVA showed significant differences between the three main ecoregions that were incorporated in this study (F=8.185; p= 0.003 and F=5.390; p=0.015 respectively). Tukey's post-hoc tests showed that only the differences between Tucuman-Bolivian forests and Inter-Andean Dry forests were significant in this respect. The exact results can be seen in Figure 13 and found in the appendix.



Figure 13. Mean total AGB per ecoregion for equation 2 and 6.

The same analysis was also carried out for the 4 forest type classes that were used in the research design, the difference with the ecoregions approach being that now Tucuman-Bolivian forest is split up in evergreen and deciduous forest. Again, for both Eq. 2 and 6, the differences turned out to be significant (F=6.058; p=0.005 and F=3.722; p=0.032). In this case, post-hoc tests showed that only the difference between evergreen Tucuman-Bolivian forest and deciduous Inter-Andean Dry forest is



significant. This can be seen in Figure 14. For Tucuman-Bolivian forest, the distinction between evergreen and deciduous forest is not found to produce a significantly different result.

Figure 14. Mean total AGB per forest type class (EG: evergreen; D: deciduous).

Conducting a factorial ANOVA with forest type class and elevation as independent factors showed that there is a significant interaction effect between these two factors as well (F=16.093; p=0.000, and F=7.862; p=0.006, respectively). However, in order to better understand this interaction effect with elevation, a multivariate analysis needs to be carried out. This will be done in Part 3.3.

From the figures, it is clear that the variation within the ecoregions or forest type classes is relatively large. Standard deviations are especially large in the case of Tucuman-Bolivian forests and Chaco Serrano. For forest types, deciduous Tucuman-Bolivian forest does not have a large standard deviation, but evergreen TB forest does. This suggest that within this group, other factors, like elevation could play a role. For the other classes, this doesn't seem to be the case.

Next, we can also study at the differences per ecosystem type as identified by Navarro and Ferreira (2007) and described in Table 1. Again, a one-way ANOVA was carried out for the 5 ecosystem types that are most important. In all cases, data was found to be normally distributed. However, for the 2 equations that were under study, Eq. 2 did not obey normality, but Eq. 6 did (p=0,041 and p=0,082 respectively). However, the results are the same in both cases: there is a significant difference between the 5 groups (F=4.444; p=0.018 and F=3.804; p=0.029 respectively).

Tuckey's post-hoc tests revealed that this significant difference is only the case between CES409.211 and CES409.207 (p=0.016 and p=0.038, respectively). This is the difference between *Inter-Andean* - *sub-Andean Tucuman-Bolivian xerophile forest* and *Sub-humid Tucuman-Bolivian forest of the upper sub-Andean region*, in other words, between the Inter-Andean Dry forest and the Tucuman-Bolivian forest in Tocopampa and Bicoquin. These are the groups with respectively the lowest and highest AGB values in the entire area. These results also confirm the significant differences that were found above for Tucuman-Bolivian and Inter-Andean Dry forests ecoregions.

Further including the ecosystem types with only 1 sample, and then running a non-parametric Kruskal-Wallis test also showed that the distributions across the 8 groups are not the same (p=0.026 and p=0.046, respectively). The differences are visualized in Figure 15, and exact figures can be found in the appendix.



Figure 15. Mean total AGB per ecosystem type for equation 2 and 6. Standard deviations are shown where possible. For details of CES-codes, see Table 1.

Including elevation as a covariate in the one-way ANOVA with the 5 main ecosystems, the overall result is similar. After adjustment for elevation there are significant differences between the 5 ecosystems, in both cases of allometric equations. However, pair-wise comparisons show that this is only the case between CES409.211 and CES409.207 (p=0.040 and p=0.019), and CES409.211 and CES406.238 (p=0.006 and p=0.013). This means that after adjusting for elevation, we not only find significant differences between IAD Forest and TB forest in Tocopampa and Bicoquin, as found before, but also between IAD forest and Chaco Serrano in Laja Toco. This suggests that elevation plays a differentiating role in AGB estimates. In order to understand better how this role can best be used in region-wide AGB estimates, a multivariate analysis needs to be conducted.

3.2.2 Biomass Distribution across Forest Compartments

The relative contribution of different forest compartments to total aboveground biomass was analysed for each site and can be seen in Figure 16. Figure 17 shows the average distribution across all 21 plots. The exact figures can be found in Table 6.



Figure 16. Distribution of aboveground biomass across 5 forest compartments.



Figure 17. Relative distribution of AGB across forest compartments, averaged over all 21 plots.

As can be seen, almost 80 per cent of all aboveground biomass is comprised of big living trees. Furthermore, it can be seen that the second-biggest contributor to AGB is plant litter, grasses and herbaceous vegetation, which comprises almost 10 per cent on average. On average, this carbon stock compartment contributes more to AGB than small living trees or necromass, although this varies per location. Necromass was found to contribute 7 per cent on average, while the small living trees seem neglectable with 2.5 per cent. This is probably because of the choice to limit this compartment to DBH a class of 2.5-5 cm only.

Site name	Big living trees (DBH ≥ 5)	Small living trees	Dead trees (DBH > 2.5)	Plant litter, grasses, and herbaceous	Lianas
		(DBH 2.5-5)		vegetation	
Pampa Negra	60,99	6,51	8,70	23,80	0
Molleaguada	80,42	5,48	5,35	8,75	0
Laja Toco	77,75	1,03	11,50	5,19	4,5
Algodonales	74,53	2,78	9,40	9,85	3,44
Тосоратра	85,90	0,49	2,05	9,01	2,55
Bicoquin	80,59	1,38	10,93	5,93	1,16
Chiriguanañan	84,35	1,53	6,22	7,58	0,33
Salsipuedes	90,88	0,97	2,64	4,59	0,92
Grande					
Average	79,43	2,52	7,10	9,34	1,62

Table 6. Biomass distribution (in %) across 5 forest compartments.

A one-way ANOVA shows that there are significant differences between the 8 sites in terms of the contribution of big living trees to total AGB (F=4.177; p=0.013). Tuckey's post-hoc tests show that this is only the case for the individual difference of Pampa Negra with respect to Chiriguanañan, Molleaguada, Bicoquin and Salsipuedes Grande. This is probably the case because in Pampa Negra, trees are substantially smaller than in other locations, while the contribution of (thorny) grasses and herbaceous vegetation is considerably larger. Without this effect, the differences between locations do not seem to be significant.

3.2.3 Spatial Variation in Forest Structure

The second part of second sub question refers to spatial variation in forest structure. Forests in the ANMI RG-VC area do not only differ in amount of stored biomass, there are also strong differences in forest structure characteristics. One of the most striking differences can be found for basal area. The Kruskal-Wallis test showed significant differences between the various communities (p=0.018). Individual post-hoc tests could not be performed because of the non-parametric character of the test, but differences in basal area are likely with respect to Bicoquin, which shows a much higher value in Figure 18. This is not surprising as Bicoquin harbours the densest form of evergreen Tucuman-Bolivian forest in the entire data set.



Figure 18. Variation in basal area across the 8 sites.

Total stem density was not found to be significantly different, but stem density within DBH classes 2.5-5, 20-30, and 60-70 cm and stem density of lianas did show significant differences between the 8 sites (p=0.009; p=0.019; p=0.034; and p=0.015, respectively). See Figure 19. This is partly the reason for the higher basal area in the case of Bicoquin.



Figure 19. Variation in stem density of various DBH classes across the 8 sites.

Mean wood density was also found to be significantly different (p=0.023), see Figure 20. Furthermore, biomass of various DBH classes also showed to be significantly different (Figure 21). This was the case for biomass in DBH class 2.5-5 (without lianas; p=0.009 for both equations), DBH 20-30 (p=0.019 and p=0.023, respectively), and DBH 60-70 cm (p=0.028 for both equations). These are the same DBH classes as those that showed significant variation in stem density. This indicates that DBH classes 2.5-5, 20-30 and 60-70 seem to contribute most to AGB variation across the region, due to variation in the amount of trees in these classes. This has in turn an effect on variation in total basal area as well.



Figure 20. Variation in mean wood density across the 8 sites.



Figure 21. Variation in biomass of various DBH classes across the 8 sites, for Eq. 2 only.

When analysed per forest type class or ecoregion, most of the DBH classes and variables turned out to not obey homoscedasticity. For this reason, ANOVA analyses per ecoregion or forest type were not performed on this data.

However, non-parametric Kruskal-Wallis tests showed that none of the variables differs significantly per type of ecoregion. This indicates that the differences between sites are more pronounced. In terms of forest structure, the three distinct ecoregions are a much too broad aggregate, because there is a lot of heterogeneity within ecoregions. This can be seen in Figure 22 for example, where stark differences between Tucuman-Bolivian forest sites are clearly visible. In this case, when analysing forest structure, it makes more sense to follow the ecosystem classification, instead of the ecoregion classification.



Figure 22. The relative distribution of AGB across DBH classes for each site.

3.2.4 Spatial Variation in Species Composition

The third aspect that was analysed in order to answer the second sub question was the spatial variation in species composition. In the 21 forest plots, a total number of 2479 individual big living trees (DBH \geq 5 cm) were measured, containing exactly 200 distinct plant species, of which 1 plant species could not identified on species or genus level, while 16 others could not be identified on species, genus or family level. Excluding these 17 unknowns, the total dataset comprised 183 distinct plant species, in 124 distinct genera, across 56 distinct families.

When analysing the abundance of each family for the entire dataset, the result resembles a logistic curve like in Figure 23. Strikingly, the most abundant plant family over all 21 plots is the Cactaceae family. This is due to the fact that many branches of the same cactaceous tree were often counted as individual stems in Pampa Negra and Molleaguada. This causes an unreliable abundance figure for Cactaceae. Thus, in fact, the most abundant families in our dataset are Fabaceae, Lauraceae, Myrtaceae, and Podocarpaceae. Looking at the same figures for biomass adjusted importance value indices (IVI-B) in Figure 24, we find the same families on top. But this time, we find that the Anacardiaceae are much more important as well, due to the presence of a couple of Anacardiaceae members that have a relatively large contribution to biomass in dry and deciduous ecosystems, such as Cuchi (*Myracrodruon urundeuva*) or Quebracho blanco (*Schinopsis marginata*).



Figure 23. Total abundance per family (\geq 10).

Figure 24. Total IVI-B per family (\geq 10).

When analysing species compositions at site level, we find the results as in Table 7. From this, it is clear that there is quite some variation in species diversity (# of unique species) per site location. This will be further analysed in Part 3.3.

The number of hyperdominant species for each site (those that together contribute more than 50 per cent of biomass) ranges from 2 to 6 species. On average, 10.5 per cent of all species in the dataset is found to be hyperdominant, but this figure is much lower in Bicoquin and Laja Toco were only ca. 5 per cent of all species contribute to more than 50 per cent of biomass. These are the sites that show most hyperdominance.

Site name	Total # of	Mean # of	# of hyper-	Relative # of
	unique	species per plot	dominant	hyper-dominant
	species		species	species (%)
Pampa Negra	24	14	3	12.5
Molleaguada	31	14	3	9.7
Laja Toco	43	22	2	4.7
Algodonales	45	22	6	13.3
Тосоратра	35	18 *(DBH≥10)	5	14.3
Bicoquin	40	25 *(n=2)	2	5.0
Chiriguanañan	24	15	3	12.5
Salsipuedes Grande	11	11 *(n=1)	2	18.2
Total (21 plots)	200	-	21	10.5

Table 7. Various characteristics of species composition.

When looking at the most characteristic species for each site (Table 8), there appears to be much diversity between the different sites as well. For each site the most important species are all very different. These results seem to correspond reasonably well with the known characteristics of each ecosystem type. According to Ibisch and Mérida (2003), Inter-Andean Dry forest is generally characterized by the presence of *Schinopsis haenkeana* (\equiv *S. marginata*) and *Aspidosperma quebrachoblanco* above 2300 meters, with partial presences of *Neoraimondia herzogiana* and other columnar Cactaceae. This corresponds with the 1st and 2nd most prominent species in Molleaguada, as well as the 3rd and 4th most prominent species in Pampa Negra.

For Tucuman-Bolivian forest, the list of most prominent species is also reasonably correct according to the characterization of this type of forest. Ibisch and Mérida (2003) mention the presence of (semi-) deciduous forest with *lapacho* in lower areas (<2200 m), which corresponds with the presence of *Handroanthus impetiginosus* (known as *lapacho rosado*) in Tocopampa and *Handroanthus lapacho* in Bicoquin. Above 2200 m, one can encounter evergreen forest with strong presence of pine trees (*Podocarpus parlatorei*), which corresponds with the presence of this species in Chiriguanañan and Salsipuedes Grande. The latter location also shows strong presence of *Alnus acuminata*, which is more characteristic of deciduous forests at these elevations. Carretero (2005) also mentions the presence of *Blepharocalyx salicifolius* in TB forest, which is found in Chiriguanañan as well.

For Chaco Serrano, Ibisch and Mérida (2003) do not mention specific species, only the presence of deciduous vegetation. However, Carretero (2005) mentions *Anadananthera colubrine* as a characteristic species, which corresponds with the most prominent species found in Laja Toco.

Overall, the encountered forest physiognomies of most of the 8 sites thus correspond reasonably well with the expectations based on the vegetation characteristics of each ecoregion. For this reason, it seems safe to assume that the obtained data is representative of the various ecoregions and can be used for extrapolation to larger areas.

Table 8. List of most important species of each site. Ranking is based on IVI-B, summed over all local plots at that site.

Site name	1 st Species	2 nd Sp.	3 rd Sp.	4 th Sp.	5 th Sp.
Pampa Negra	Browningia caineana	Cardenasio- dendron brachypterum	Schinopsis marginata	Neoraimondia herzogiana	Cynophalla retusa
Molleaguada	Schinopsis marginata	Aspidosperma quebracho- blanco	Pterogyne nitens	Piptadenia boliviana	Browningia caineana
Laja Toco	Anadananthera	Myracrodruon	Phyllostylon	Stillingia	Parapipta-
	colubrina	urundeuva	rhamnoides	tenella	denia excelsa
Algodonales	Ocotea	Pachystroma	Cinnamomum	Recordia	Unknown
	Iongifolia	Iongifolium	triplinerve	boliviana	(Alg.6)
Tocopampa	Recordia boliviana	Handroanthus impetiginosus	Myracrodruon urundeuva	Machaerium acutifolium	Anadenan- thera colubrina
Bicoquin	Parapiptadenia	Handroanthus	Licaria	Machaerium	Ocotea
	excelsa	Iapacho	triandra	Iatifolium	minarum
Chirigua-	Podocarpus	Blepharocalyx	Myrcianthes	Symplocos	Prunus
nañan	parlatorei	salicifolius	pseudomato	neei	tucumanensis
Salsipuedes	Podocarpus	Alnus	Sebastiania	Viburnum	Vallea
Grande	parlatorei	acuminata	ovata	seemenii	stipularis

3.3 Multivariate and Regression Analysis

With the factorial ANOVA that was performed, we have seen that there are multiple factors that have individual or combined effects on stored biomass. We have also seen that there is considerable variation in species composition and diversity across the 8 selected sites. In order to further analyse this and link this to possible environmental variables, a multivariate analysis was conducted. This will help to address the third sub question, which read: *Which stand or environmental factors contribute most to regional spatial variation in AGB and species composition, and how can these be used for mapping regional AGB values*?

3.3.1 Multivariate Ordination Analysis of Species Composition and Diversity

Firstly, a multivariate analysis of variation in species composition and diversity was performed using various ordination techniques. In this way, all the variation in the dataset could be studied and related to possible stand or environmental predictor variables through principal axes.

Via unconstrained ordination in the form of a detrended correspondence analysis (DCA), the total variation in the plant species dataset was first analysed. The total variation was found to be 9.83 points on the IVI-B scale. The first four principal axes that were found cumulatively explained 25.77 per cent of the total variation in this data. This low figure indicates that there are various principal factors involved in species variation. Of these 4 axes, the first one explained 10.02 per cent of the variation, while the second one added another 8.07 per cent to that, totalling 18.09 per cent. Table 9 shows these results, together with the results of other tests to be explained below.

Ordination Type	Ordination Method	Explanatory Variables (#)	Total variation explained (%)	Cumulative explained variation (%)			
				Axis 1	Axis 2	Axis 3	Axis 4
Unconstrained	DCA	-	-	10.02	18.09	22.40	25.77
Constrained	CCA	Environmental (12)	72.9	9.96	18.85	27.18	34.88
Constrained	CCA	Stand (3)	24.5	9.87	18.69	24.45	34.44
Constrained	CCA	Environmental + Stand (15)	84.2	9.99	18.90	27.28	35.36
Constrained	CCA	Environmental (4)	36.4	9.70	18.06	25.49	32.47

 Table 9. Overview of ordination results.

Figure 25 shows the location of each of the 21 forest plots with respect to these 2 principal axes. As can be seen, plots from the same site cluster very well together into 8 different groups. This indicates that the species compositions of the multiple plots at each site are very similar.



Figure 25. Detrended correspondence analysis (DCA) of species composition of the 21 forest plots. Colours indicate the number of unique species per plot, ranging from 9 (green) to 32 (dark blue) species. Red circles indicate plots from the same site location.

What further can be seen is the strong separation of Inter-Andean Dry forest (plots 7, 8, 9 and 14, 15, 16) from the rest of the plots, on the horizontal axis. Chaco Serrano (plots 11, 12, 13) is then positioned in between IAD and the Tucuman-Bolivian forest plots on the left side.

On the vertical axis, we see a separation between Algodonales and Tocopampa in the upper left corner, and Chiriguanañan and Salsipuedes Grande in the lower left corner. This is a separation of the lowerelevation plots and the higher-elevation plots. The vertical axis thus seems to be related to elevation. The different colours of the dots in Figure 25 indicate species diversity. Green dots have a low number of unique species, while blue dots are high in species diversity. From this, it can be found that species variation is highest in plot 5 in Algodonales, followed by plot 17 in Bicoquin, and 11 and 13 in Laja Toco. Salsipeudes Grande shows relatively little species variation, as do Chiriguanañan and the Inter-Andean Dry forest locactions. This corresponds with the mean number of unique species found in Table 7.

A constrained analysis was also carried out on the same dataset in the form of a canonical correlation analysis (CCA), which 12 environmental variables. In this case, it was found that these 12 explanatory variables account for 72.9 per cent of the total species variation in the dataset (pseudo-F=1.8; p=0.002). This time, the first axis explains 9.96 per cent of the variation, and the second one another 8.89 per cent, totalling 18.85 per cent (Table 9). Surprisingly, this is a slightly higher value than in the unconstrained DCA analysis, where one would expect more variation since the data is unconstrained. This might be due to the high number of explanatory variables, which makes the CCA analysis less accurate.

Only applying the CCA analysis with the 3 stand variables results in a total explained variation of 24.5 per cent (pseudo-F=1.5; p=0.002). For this, data from Tocopampa was excluded, as those stand variables are not comparable with the rest of the dataset. In this case, the first axis explains 9.87 per cent of the variation, and the second one 8.82 per cent, totalling 18.69 per cent (Table 9).

Together, stand and environmental variables explain 84.2 per cent of the total variation found in the dataset, as can again be seen in Table 9 (pseudo-F=1.8; p=0.002).

Figure 26 shows the resulting ordination diagram for the CCA with 12 environmental variables. Environmental variables are indicated by red arrows, and the 21 forest plots by circles. The direction of the arrows is related to the maximum correlation with the 2 principal axes, while the length of the arrow is related to the strength of the correlation.

Once again, we find a large separation between the Inter-Andean Dry forest sites on the one hand, and the Tucuman-Bolivian forest and Chaco Serrano sites on the other hand. The latter group is spread out across the vertical axis, while the separation between IAD sites and the other locations takes place on the horizontal axis. According to the environmental variables most in line with the axes, this horizontal axis represents an effect of drought stress, with increasing environmental stress (E) to the right, and thus increasing precipitation in August (Prec8) and increasing variation in NDVI (NDVI Dif 2) to the left. On the vertical axis we find a main effect of elevation (DEM), which is related to an opposite effect of mean annual temperature (AnTemp) and various other climatic variables.



Figure 26. Ordination diagram (CCA) of species composition of the 21 forest plots, with 12 environmental variables. Species diversity of each plot is indicated by the size of the circle.

From this, we can conclude that the presence of a strong dry season (drought stress) has an impact on species composition for Inter-Andean Dry forest, but not so much for Tucuman-Bolivian forest or Chaco Serrano. The differences in species composition for the latter group are primarily caused by the elevation gradient. For Inter-Andean Dry forest, elevation does not have an effect.

When we select only these 5 environmental factors that are most related to the principal axes for further CCA analysis (Figure 27), the predictive power is less: together these 5 variables account for 36.4 per cent of total variation (pseudo-F=1.7; p=0.002). This means that there is some collinearity effect between these 5 factors and the other 8, since the total explanatory power is lower. The cumulative explained variation by the 2 principal axes is 18.06 per cent. This figure is lower than that of the unconstrained DCA, as expected, indicating that this CCA with only 5 variables is probably more accurate than the one with 12.

Figure 28 shows the ordination diagram belonging to the CCA with only the 3 stand variables. This is an altogether different result. Clearly, no logical principal axes can be deduced from this as all plots are scattered out and the explanatory variables are not directly aligned with one of the axes. This indicates that the explanatory power of the stand variables stems from the fact that they are correlated to the environmental variables as discussed above.



Figure 27. Ordination diagram (CCA) of species composition of the 21 forest plots, with the 5 most important environmental predictors. Species diversity of each plot is indicated by the size of the circle.



Figure 28. Ordination diagram (CCA) of species composition of the 21 forest plots, with the 3 stand variables. Species diversity of each plot is indicated by the size of the circle.

3.3.2 Multivariable Regression Analysis of Aboveground Biomass

A multivariable analysis was also applied to analyse the variation in AGB (according to Eq. 2) across the 21 forest plots. This allows us to possibly relate spatial variation in AGB to stand or environmental factors and use this in a predictive way via a regression model. This analysis could not be done in the form of ordination analysis (*multivariate* analysis), as there is only 1 dependant variable (total AGB). Instead, a *multivariable* generalized linear model (GLM) was applied. This type of linear regression allows us to model AGB based on multiple independent variables that are not necessarily normally distributed. The GLM regression showed that the 12 environmental variables explain about 92.3 per cent of all variation in AGB following a Gaussian distribution with identity-link function (pseudo-F=8.0; p=0.004). In order of strength, the three strongest predictor variables in this case are: total precipitation of the 3 driest months (p(T)= 0.621), environmental stress variable *E* (p(T)=0.471), precipitation seasonality (p(T)= 0.446).

Redundancy analysis (RDA) of the variation in AGB was also performed as a function of the 3 main stand variables. This was done for the 18 plots without Tocopampa. These three stand variables explain 97.1 per cent of the total variation in AGB (pseudo-F=154; p=0.002).

However, stand variables cannot be used for landscape-scale modelling of AGB, as there are no regionwide base maps of stand variables available. The generalized linear model with 12 environmental variables is not useful either for this purpose, because of its complexity. For modelling purposes, a simpler general linear model with less variables is required. For this reason, it was decided to continue with further step-wise multiple linear regression analyses in SPSS, instead of generalized linear models in CANOCO.

Performing a step-wise multiple linear regression for all 21 sites with all 12 environmental variables resulted in an overall best model consisting of average August precipitation (P8) and precipitation seasonality (PS), with an adjusted R² of 0.55 (F=13.246; p=0.000):

$$AGB\left(\frac{t}{ha}\right) = -1006,143 + 22,536 * P8 + 10,563 * PS$$
 (Eq. 12)

This shows that the main variation in AGB between IAD and TB and CS sites is due to the influence of the dry season in IAD, of which August precipitation and precipitation seasonality are strong indicators. This follows the same conclusion for species diversity that differences between IAD and TB and CS forest are due to the drought stress.

However, this regressions has a relatively low adjusted R², which makes it unreliable for use in extrapolation over the entire IAD, TB and CS forest areas. This is because the dataset features relatively many Tucuman-Bolivian forest sites, and only few Inter-Andean Dry forest or Chaco Serrano sites.

For this reason, it was decided to apply the same step-wise multiple linear regression analysis only to Tucuman-Bolivian forest plots. This results in the following regression equation for AGB:

$$AGB\left(\frac{t}{ha}\right) = -357,272 - 0,810 * CWD - 1,435 * PDQ$$
 (Eq. 13)

Here the strongest predictor variable is the climatic water deficit CWD, followed by the average total precipitation of the driest quarter (PDQ). This regression accounts for 95.5 per cent of the AGB variation in the Tucuman-Bolivian forest ecoregion (adjusted R²=0.955; F=117.377; p=0.000). This result shows that, contrary to what was hypothesized, elevation does not have an effect on overall AGB within this ecoregion. Instead, drought stress, as measured by CWD and PDQ, does.

Conducting the same analyses with results from allometric equation 6 instead of 2, we get the following regression equation for Tucuman-Bolivian forest (adjusted $R^2 = 0.893$; F=92.722; p=0.000):

$$AGB\left(\frac{t}{ha}\right) = -841,128 - 1,448 * CWD$$
 (Eq. 14)

Contrary to the case with Eq.2, only CWD is here sufficient enough to explain obtain a reliable adjusted R² of 0.893. Hence, both models can reliably be used for modelling of AGB data in the Tucuman-Bolivian forest ecoregion.

3.4 Mapping Aboveground Biomass in the ANMI RG-VC

The second part of the third sub question asked how stand or environmental predictor variables can be used for the mapping of regional AGB values. As has been mentioned, stand variables are not suitable for this, as there exist no region-wide base maps of these variables. However, with the results obtained in the previous parts, various ways of mapping AGB based on environmental data can be conceived.

Firstly, the simplest map would be based on the average AGB values of the 3 main ecoregions that were studied. We can create a map with only these average values per region. For Tucuman-Bolivian forest this is 201.19 t/ha, for Chaco Serrano 178.37 t/ha, and for Inter-Andean Dry forest 78.15 t/ha (based on Eq. 2). In this map, a value of 192.49 t/ha can be used for the Gran Chaco region that was not included in this study (Arnstein 2014). For the Yungas region, a value of 303.81 t/ha can be used, based on data in the municipality of San Carlos (Linneo 2014), which probably is the most representative of all data available. The resulting aboveground biomass map then looks like Figure 29. When summing this over the total forested areas, the total AGB value for the entire ANMI RG-VC area becomes 108.08 Mt of biomass, or on average 178.71 t/ha of forest.

Since no significant difference was found between evergreen and deciduous Tucuman-Bolivian forests, splitting this map into the different forest type classes does not improve these results. In terms of mapping AGB on landscape-scale, the forest type class approach is not more accurate.



Figure 29. Aboveground biomass distribution in the ANMI RG-VC, based on ecoregion averages.

In a similar way, a biomass map can also be created based on the average ecosystem values, as in Figure 30. Average AGB values for the 8 main ecosystem are shown (based on Eq.2). The result is a total aboveground biomass value of 95.02 Mt for these 8 ecosystems (94.4 per cent of total ANMI RG-VC area), or on average 163.66 t/ha of forest. When assuming that the remaining 5.6 per cent area of the ANMI RG-VC is Tucuman-Bolivian forest, with on average 78.3 per cent forest cover, we get a total AGB value of 101.26 Mt, or 165.56 t/ha of forest. These figures are thus slightly lower than those of the previous map, by ca. 10 per cent.



Figure 30. Aboveground biomass distribution in the ANMI RG-VC, based on the main ecosystem averages.

Applying Eq. 13, the developed regression model for the Tucuman-Bolivian forest ecoregion (based on allometric equation 2), we get an average of 183.22 t/ha for Tucuman-Bolivian forest, ranging from 57 to 370 t/ha as extreme values. This average is thus lower than the TB plot average of 201.19 t/ha (ca. 10 per cent difference), indicating that the current plot distribution might be more skewed towards high-biomass forests. The modelled distribution of biomass can be found Figure 31. Together with the plot averages of the other 4 ecoregions, this gives a total of a 104.18 Mt for the entire ANMI RG-VC, with on average 172.27 t/ha of forest.



Figure 31. Aboveground biomass distribution in the Tucuman-Bolivian forest ecoregion, based on regression model Eq. 13. Values in tonnes/ha.

Applying Eq. 14, the regression model for Tucuman-Bolivian forest based on allometric equation 6, we get an average of 247.24 t/ha of TB forest, which ranges from 39 to 514 t/ha as extreme values. See Figure 32 for its distribution. This figure is thus much higher than the plot average TB value. Together with the averages of the other regions, this gives a total of a 118.06 Mt for the entire ANMI RG-VC, with on average 195.22 t/ha of forest.



Figure 32. Aboveground biomass distribution in the Tucuman-Bolivian forest ecoregion, based on regression model Eq. 14. Values in tonnes/ha.

Comparing the differences between the three approaches for TB forest, we can note the following (see Table 10). In case of allometric equation 2, the difference between using a plot average or a regression approach is ca. 10 per cent (183.22 and 201.19 t/ha). The difference between allometric equation 2 and 6 in the case of a regression model is much larger, though, ca. 35 per cent (183.22 and 247.24 t/ha). This is consistent with the large differences found in the earlier methodological analysis.

5				
Approach	Allometric Equation	Mean AGB	Lowest AGB	Highest AGB
		(t/ha)	(t/ha)	(t/ha)
Plot average	2	201.19	-	-
Linear Regression Model	2	183.22	57	370
Linear Regression Model	6	247.24	39	514

Table 10. Overview of results from different mapping approaches for the Tucuman-Bolivian forest ecoregion.

4. Discussion

The main research question of this research was as follows: *What is the influence of environmental factors on regional AGB estimates for avoided deforestation projects in the tropical dry forests of the Río Grande - Valles Cruceños Natural Area of Integrated Management, and how is this related to forest structure and species composition?* In order to answer this, this research focused on three aspects, namely the uncertainty of certain methodological choices for estimating AGB, the variation in AGB, forest structure and species composition at various spatial levels across the ANMI RG-VC area, as well as the predictive power of stand and environmental factors on AGB and species composition for modelling purposes. In this part, the results of these three aspects will be discussed in light of the main research question and their application for avoided deforestation projects as implemented by Fundación Natura Bolivia.

4.1 Comparison of Obtained AGB Values

Firstly, we will look at the AGB values obtained in the second part of the results section. In order to assess the correctness of these values, we can compare them with values from other studies. For the main ecoregion of Tucuman-Bolivian forest, this study obtained a value of 201.2 t/ha based on 5 different plot sites. No significant difference was detected for sites classified as evergreen or deciduous forest based on RapidEye remote sensing data. This value can be compared with similar forest types of evergreen to semi-deciduous Amazon forest in Bolivia. In most cases, these studies used DBH \geq 10 cm as the lower measurement limit. However, this is still comparable as the 5-10 cm DBH class generally only forms a small fraction of total AGB in these kinds of forest. Araujo-Murakami *et al.* (2014) found values of 144,3 t/ha for dry Amazon forest (transition between humid Amazon and Chiquitanía transition forest), while Araujo-Murakami *et al.* (2006) found 207,9 t/ha for dry semi-deciduous forest in the Noel Kempff Mercado National Park. Following a different methodology, Dauber *et al.* (2000) found 191 t/ha for pre-Andean Amazon forest. Thus, except when compared with Araujo-Murakami *et al.* (2014), the outcome of this study for Tucuman-Bolivian forest is reasonably in line with the results of Araujo-Murakami *et al.* (2006) and Dauber *et al.* (2000).

For Chaco Serrano, we can compare the value found in this study (178.4 t/ha) with that of the Gran Chaco as obtained by Arnstein (2014). She found a total AGB value of 192.5 t/ha in the community of Yumao, in the southern part of the ANMI RG-VC. Not much other data on Chaco Serrano forests exists, except for the estimations by Dauber *et al.* (2000), who calculated 157 t/ha of AGB for Chiquitanía forest, and 133 t/ha for the Chiquitanía-Amazon transition forest. These values are lower than what was found in this study. However, it would be reasonable to expect a higher value of AGB in Chaco Serrano than in Gran Chaco forest, since this type of forest is often wetter, denser and features taller trees. This is not the case, which might be due to the difficulties in correctly classifying Chaco Serrano and Gran Chaco areas in the ANMI RG-VC, as well as because of the different methodologies applied.

In a comprehensive study of the entire Amazon basin, Saatchi *et al.* (2007), stated that the Central Amazon generally holds > 300 tonnes AGLB / ha (excluding necromass), while in the Western Amazon this value drops to between 150-300 tonnes of AGLB per hectare. For humid, evergreen Amazonian forest, literature suggests various values of AGB as well. According to Dauber *et al.* (2000), Amazonian forest contains 228 t/ha. However, others report much higher values, such as Araujo-Murakami *et al.*

(2006) for humid, evergreen forest in the Noel Kempff Mercado National Park: 260,6 t/ha. Hence, values of 202 and 178 t/ha for tropical dry forest types such as Tucuman-Bolivian forest and Chaco Serrano, as obtained in this study, seem to correspond reasonably well with the expectations set by these sources.

No specific data is available for Inter-Andean Dry forest, so the obtained value of 78.2 t/ha AGB cannot be compared in this case. It must be noted that this value is significantly lower than the other values, as was expected since IAD forest features much sparser and lower vegetation. However, Gran Chaco is generally considered to harbour even lower vegetation than Inter-Andean Dry forest. Hence, there is a striking difference between the low IAD value obtained here, and the high Gran Chaco value of 193 t/ha as reported by Arnstein (2014). Again, this might be due to the difficulties in correctly classifying the various – highly related – ecoregions. One explanation could be that the forest as sampled by Arnstein (2014) is actually more representative of Chaco Serrano than of Gran Chaco, which correlates with the 178 t/ha of AGB in Chaco Serrano that this study found.

4.2 Sources of Error

As has become clear above, differences in AGB estimates can be large. One aspect that could have an influence on this are the differences in field methodologies. Hence, part of a proper approach to estimating tropical biomass is understanding the correctness of the methodology applied. For this reason, the first sub question of this research was as follows: *What are the effects of different methodological choices on estimated AGB values?* This question was specifically addressed because this study made some uncommon choices in the design of its field method, such as a lower DBH limit of 5 instead of 10 cm and a plot size of 0.1 instead of \geq 0.25 ha. The effects of these 2 choices were analysed, together with the effect of the choice of allometric equation. 7 allometric equations were applied, of which the first 5 were taken from various sources, while Eq. 6 and 7 were adaptations from Eq. 4 and 5. Instead of a normal estimation of tree height by eye, these last 2 equations estimated tree height allometrically based on the tree's diameter. It was found that this difference had the biggest effect on final outcomes, with ca. 40-45 per cent increase for Eq. 6 and 7 with regards to Eq. 4 and 5. However, independent of this, the choice of allometric equation itself, i.e. between Eq. 1-5, has a large effect on estimated AGB as well, with ca. 20-35 per cent differences in final outcome.

No significant differences between Eq. 2 (Brown 1997), Eq. 4 (Chave *et al.* 2005, with tree height) and Eq. 5 (Chave *et al.* 2014) were found. Eq. 2 only includes tree diameter and wood specific density as variables, while the other two equations also include tree height. However, Eq. 6 and 7, which are the adaptations of 4 and 5 with allometric estimation of tree height following Feldpausch *et al.* (2012), produce much larger values of forest biomass. This seems to indicate that this latter approach results in a significant overestimation of AGB. Since Eq. 2, 4 and 5 have different structures and are designed by different authors, yet still produce the same result, they seem to be the most reliable.

Of these three, Eq. 2 does not rely on tree height at all, yet produces the same results as Eq. 4 and 5. For this reason, it seems to produce the most reliable estimate of total AGB, in the simplest way. The results form Eq. 2 were also the only of the first 5 equations to be normally distributed. For this reason, this equation by Brown (1997) has been applied as the most reliable estimate to date for the ANMI

RG-VC region. All regional AGB values mentioned are based on this equation, unless specified otherwise.

Eq. 3 (Chave *et al.* 2005, without tree height) was the most different from the others equations (1-2 and 4-5). This corresponds with the findings by Alvarez *et al.* (2012), who stated that this version of the Chave *et al.* (2005) equation (without tree height) can systematically overestimate aboveground biomass. They concluded that Chave *et al.*'s forest classification (2005) might not produce the best results. For this reason, it could be that the 'dry forest' formulas by Chave *et al.* (2005) are not very applicable to the tropical dry forest in the ANMI RG-VC region, especially when altitudinal and other complex environmental gradients are present (Alvarez *et al.* 2012). This is another reason to prefer Eq. 2 over the others by Chave *et al.* (2005, 2014) as the most reliable estimate.

Another uncertainty factor within any field inventory methodology is the choice of plot size. For this reason, the mean result of three small 0.1 ha plots was compared with the result of a larger 1 ha plot, for the same Tucuman-Bolivian forest area in Tocopampa. The difference between these methods turned out to be small, ca. 10 per cent. The values of the 1 ha plot are consistently within the error margins of the three 0.1 ha plots, and the effect seems negligible. The paired-samples t-test was found to be significant, though this test is not entirely valid in this case. For this reason, it appears correct to conclude that, at least for Tucuman-Bolivian forest, the choice of 0.1 ha plot size is no large source of error. This strengthens the decision to install multiple smaller forest plots, instead of a few large plots, as suggested by Chave et al. (2004) and Baraloto et al. (2013). Especially in topographically and ecologically heterogeneous terrain like the ANMI RG-VC, such an approach is preferable, since the effect of plot representativeness on a landscape-scale is likely to be a larger source of error (Chave et al. 2004). However, more research on this is needed. The same authors that recommend using multiple smaller plots, warn against using smaller than 0.25 ha forest plots (Chave et al. 2004; Baraloto et al. 2013). Yet, it could be that these feared error effects depend very much on the type of forest under investigation. For example, Inter-Andean Dry forest generally features sparser vegetation and stores less biomass. Because of this, a (few) single large individual tree(s) can have pronounced effect on the final AGB estimate. This effect could be reduced by installing larger plots. For Tucuman-Bolivian forests, this appears less of a problem. However, this effect could not be studied in this research since no 1 ha plots were installed in IAD forest. Hence, this is something that should be addressed in future research.

The third methodological choice that has been addressed is the choice between setting a lower measurement limit of DBH \ge 5 cm or DBH \ge 10 cm. This problem was addressed because a significant difference was expected for IAD forest. Most trees and Cactaceae in that ecoregion fall within the 5-10 cm DBH range. Thus, it was opted to set a lower limit of 5 cm for all plots in order to not miss out on this large biomass fraction. It turned out that this choice did not result in significant AGB differences for Tucuman-Bolivian and Chaco Serrano forest. Based on this, is can be concluded that the choice for DBH \ge 5 cm did not significantly alter the estimation of AGB in TB and CS forest, while it probably produced more accurate results for IAD forest. However, choosing the 5 cm limit is a much more labour-intensive methodology. Because of this, it is recommended to set a lower limit of DBH \ge 5 cm for any further studies in Inter-Andean Dry forest, while a limit of DBH \ge 10 cm can best be set for Tucuman-Bolivian and Chaco Serrano forest.

It needs to be mentioned though that this 5 cm limit for Inter-Andean Dry forest communities had a specific effect on forest structure characteristics. Because of this limit, IAD sites showed a specifically high stem density for small DBH classes. This is due to the fact that in these regions, vegetation is characterized by a relatively large number of small trees and cactuses with many branches. During field measurements, each branch at breast height was measured as a single stem, which is not an accurate measure for counting stem numbers, which normally only involved the main trunk. However, it was necessary to count in this way, since otherwise a lot of branches would have been neglected and overall AGB would have been largely underestimated. This highlights the fact that the current approach and allometric equations, based on diameter measurements at breast height, are not accurately applicable to shrub-like forests such as in the Inter-Andean Dry forest ecoregion. Uncertainties in small trees also tend to be larger, because the allometric equations are less applicable to these kind of diameters. Ideally, specific equations for this different kind of ecosystem should therefore be developed for future research.

Furthermore, besides these 3 sources of error, the landscape representativeness of plots was assessed as well. For this, the regional variation in AGB (between all 21 plots) and local variation in AGB (between the 3 plots at each site) were compared. It was found that the regional variation in AGB between all 21 plots is about 2.9 times higher than the average variation in local AGB between the 3 plots at each site. However, local variation between the 3 plots at each site is still pronounced, with standard deviations in the order of 10-20 per cent of the mean. Thus, even at a local level, spatially explicit sampling is much required in this kind of data gathering. This spatial variation at a local level even has a larger effect on final results (10-20 per cent) than the choice of plot size (ca. 10 per cent). On the other hand, its effect is smaller than that of the choice between allometric equations (20-45 per cent).

Together, these results indicate that error type 4 (landscape representativeness) as identified by Chave *et al.* (2004) is a big source of error for reliable AGB estimates in this kind of geographic areas, more than error type 3 (sampling size). Yet, error type 2 (allometric equation) is still the biggest source of error overall. Since error type 4 is larger than error type 3, opting for a sampling design with more representative but smaller forest plots is a reliable sampling strategy. Based on these results, it is recommended that the 3 local forest plots at each site are spread across a larger area in order to capture more landscape-scale environmental variation. In that way, each forest plot could be representative of a single forest type (e.g. a study with 21 instead of 8 sites), while plot size error is limited. This is likely to produce more accurate results than finding the means of 3 plots at a single site, which only captures 8 different sites and vegetation types in this case.

However, it must be noted that these methodological analyses are based on limited datasets, and therefore only serve as indicators of uncertainty. No fully statistically accurate conclusions based on a complete sensitivity analysis can be drawn from this. Yet it is clear that area representation is one of the most pressing issues for landscape-scale biomass estimates in heterogeneous terrain like the ANMI RG-VC.

4.3 Ecoregion and Ecosystem Averages

When extrapolating AGB data to landscape-scale, we have to take this spatial representation into account. This was further addressed through the second sub question, which read: What is the regional variation in estimated AGB, forest structure and species composition across the Río Grande - Valles Cruceños Natural Area of Integrated Management? The first aspect of this question, regional variation in AGB, can be addressed at various spatial levels. First of all, this research looked at AGB variation at both the forest class type level (ecoregions according to Ibisch and Mérida (2003), in combination with evergreen and deciduous forest classes) as well as the ecosystem level (according to Navarro & Ferreira (2007)). However, for forest class type, no significant difference in total AGB between evergreen and deciduous Tucuman-Bolivian forest was found. This distinction, as made in the remote sensing classification, therefore appears ineffective. It is a blurry distinction that does not necessarily help in classifying forest types. As could be seen in Table 2, all ecoregions and ecosystems show a mix of evergreen and deciduous forest. Even specifically deciduous ecoregions like IAD forest or Chaco Serrano show large portion of evergreen forest, which is difficult to account for in the field. This distinction hence does not appear to be very accurate. For this reason, it is recommended to neglect this distinction in further landscape-scale analyses of biomass for avoided deforestation projects. Instead, research could be done at the ecoregions and/or ecosystem level. Because of this, the distinction between different forest class types, as made in this research, turned out to be ineffective as well. Further discussion of this will henceforth only focus on ecoregions.

At this ecoregion level, only the difference in AGB between IAD forest and TB forest was found to be significant. This means that TB and CS are not significantly different in terms of biomass storage. However, the 5 different TB sites showed that there are large variations in AGB within such an ecoregion. Hence, an approach that has a higher spatial resolution seems preferable, such as the ecosystem approach, which differentiates better between different vegetation types. For future research, it is therefore recommended to set up a sampling design based on ecosystems and to try to get additional forest plots for all ecosystem types that are currently only measured by 1 forest plot. If there were 3 forest plots for each ecosystem, there would be an even better biomass estimate of the 94 per cent of the entire ANMI RG-VC that these 8 ecosystems cover.

However, when looking at total AGB values for the entire ANMI RG-VC area, by summing all the averages per class, the differences between the ecoregion approach and the ecosystem approach are not remarkably different: ca. 10 per cent only.

4.4 Modelling Aboveground Biomass

An even more accurate approach to obtain landscape-scale AGB estimates is the modelling approach. This was addressed in the third sub question, which read: *Which stand or environmental factors contribute most to regional spatial variation in AGB and species composition, and how can these be used for mapping regional AGB values?* In order to address the AGB aspect of this question, a multivariable analysis was conducted, in which two regression models were developed for Tucuman-Bolivian forest: one based on allometric equation 2, and the other on allometric equation 6. These two regression equations produced very distinct outcomes. In the case of Eq. 2, Tucuman-Bolivian forest on average stores 183.22 t/ha, while this is 247.24 t/ha in the case of Eq. 6. This is a difference of 35 per cent. This methodological difference at landscape-scale corresponds with the methodological

difference between allometric equations at plot level, which was found to be in the range of 20-45 per cent. When comparing the results from the regression model with that of the ecoregion approach, we find that the regression result (183.22) is ca. 10 per cent lower than that of the ecoregion approach (201.19 t/ha). This indicates that the current plot site distribution might be more skewed towards high-biomass forest, and is not entirely representative.

In must be mentioned, though, that the regression models produced internal AGB differences within the TB ecoregion that were even larger than based on the site results only. In the case of the second regression model (Eq. 6) highest values were more than 10 times larger than lowest values. This further confirms the large internal variation within Tucuman-Bolivian forest. For other ecoregions such as Inter-Andean Dry forest, this is not expected when applying a regression model, since these types of forest show less internal variation.

In the regression model based on Eq. 2, the drought effect (in the form of the Climatic Water Deficit in combination with the total precipitation of the driest quarter) was found to be the most important predictor variable. For the model based on Eq. 6, only CWD was sufficient to predict spatial variation in AGB. However, CWD does not model the drought effect so well for the overall area, in the case of a regression model based on all sites. In that model for the TB, CS and IAD ecoregions together, precipitation in August and precipitation seasonality are found to be the strongest predictor variables, instead of CWD.

This indicates that the seasonality of precipitation, and specifically the drought stress during the driest month, is the most important differentiating factor in AGB variation between the 3 main ecoregions, as was hypothesised. Specifically for IAD and CS forest, it is the extremities of precipitation that are a limiting factor to biomass accumulation. These findings correspond with the results by Chave *et al.* (2004) and Saatchi *et al.* (2007) who found that biomass accumulation in tropical dry forests is mostly limited by the length of dry season. On the other hand, Becknell *et al.* (2012) found that mean annual precipitation alone accounts for more than half of the AGB variation in tropical dry forests. In this study, no such relation with mean annual precipitation was found. However, within Tucuman-Bolivian forest, the year-round, overall water stress (in the form of CWD, which is a yearly deficit) is the most limiting factor to biomass accumulation. Here, the extremities have less of an impact than the year-round deficit, which corresponds with the observation by Becknell *et al.* (2012).

Elevation (or rather annual temperature) was not found to be of any significant influence on AGB. This is contrary to findings of elevation effects in tropical rain forest, such as by De Castilho *et al.* (2006), Girardin *et al.* (2014) and Réjou-Méchain *et al.* (2014). This indicates that these observations are not applicable to tropical dry forest, in which drought stress turns out to be most limiting factor.

4.5 Forest Structure

One explanation that links this drought effect to biomass accumulation was given by Malhi *et al.* (2006). They stated that the forest basal area declines with increasing dry season length, which causes lower biomass values. For this reason, the second part of the second sub question of this research addressed the regional variation in forest structure.

Significant differences in basal area were indeed found between the 8 sites, with lowest values in the driest communities of Pampa Negra and Molleaguada, and the highest value in the wettest community of Bicoquin. This confirms the ideas by Malhi *et al.* (2006). Hence, we can conclude that biomass accumulation in tropical dry forests is correlated to the dry season impact via its effect on basal area.

Further significant differences across the different research sites were found for mean wood density. The redundancy analysis found that basal area, stem density and mean wood density together explain 97.1 per cent of the total variation in AGB. This confirms previous work by Baraloto *et al.* (2011).

Furthermore, it was found that there are significant differences in the contribution of certain DBH classes to total biomass and stem density. There is a high spatial variability for these forest characteristics. For this kind of analysis, the ecosystem classification thus seems to be much more accurate. It was found that DBH classes 2.5-5, 20-30 and 60-70 contribute most to AGB variation across the region, due to variation in stem density within these classes and the resulting differences in overall basal area. This indicates that the drought effect specifically has a limiting impact on the amount of young, small trees and shrubs that can grow, as well as on the amount of very big trees that can sustain themselves.

Analysis of the biomass distribution in general showed that the big living trees (DBH \ge 5 cm) are the biggest contributor to overall aboveground biomass, with on average almost 80 per cent. However, plant litter, grasses and herbaceous vegetation, as well as necromass played a large role too, more so than small living plants (DBH 2.5-5 cm). This indicates that these compartments are important aspects to take into account when designing a nested-sampling method. Often, they are neglected in sampling methods (Baker *et al.* 2007; Baraloto *et al.* 2013). Especially better formulas for estimating dead woody biomass are much required for future research (Baker *et al.* 2007).

4.6 Species Composition

The third aspect, next to AGB and forest structure, that was addressed in the research question was species composition. The variations in species composition across the sites turned out to be even more pronounced than those of forest structure. Species composition did not vary strongly within the 3 plots of a single site, but did vary strongly between these locations across 2 environmental gradients. Contrary to what was hypothesised, it was found that elevation (or annual temperature) was the strongest differentiating factor between species composition within the Tucuman-Bolivian forest ecoregion. Drought stress (as related to precipitation in August, environmental stress factor E and NDVI differences) was a strong second differentiating factor between TB, CS, and IAD forest in general. This confirms the findings by Engelbrecht *et al.* (2007), that drought sensitivity shapes species distribution patterns. In the ANMI RG-VC, this appears particularly the case between the ecoregions. Within ecoregions (i.e. TB forest in this case), elevation instead of drought sensitivity determines species composition.

Because of this strong difference in species composition between various locations, there is also a strong difference in species contribution towards biomass accumulation. It was found that, on average, ca. 10 per cent of all the 200 species identified in this study contribute more than 50 per cent of the total aboveground biomass. For Laja Toco (Chaco Serrano) and Bicoquin (TB), this figure is close to 5

per cent. However, this is less hyperdominant than in the Amazon as a whole, where ca. 1 per cent of all the species accounts for more than 50 per cent of total biomass (Fauset *et al.* 2015). This difference is probably due to the fact that the spatial variation in species composition in the ANMI RG-VC is larger than in tropical rain forest in general.

In the ANMI RG-VC, there seems to be a hyperdominance of generally 3-5 species per site, which together contribute more than 50 per cent of total AGB at that site. These hyperdominant species are different for each of the 8 sites. Since species composition was shown to be strongly related to environmental gradients (either elevation or drought stress), the present of certain hyperdominant species is determined by these elevation gradients as well.

4.7 Linking Species Composition, Forest Structure and Biomass

However, in the multivariable analysis of AGB variation, it was found that biomass in Tucuman-Bolivian forest was most strongly related to CWD, not elevation. Hence, we find a differential effect here. For the Tucuman-Bolivian ecoregion, the presence of hyperdominant species turns out to be determined by elevation, while overall plot biomass is determined by water availability. This indicates that it is not the presence of certain hyperdominant species itself that determines the variation in AGB values within this ecoregion. Rather, it is the extent to which these species can be present in large numbers (stem density) or sizes (basal area) that determines final AGB outcomes. In this way, stem density and basal area function to mediate the influence of species composition on overall aboveground biomass, through environmental constraints such as drought stress (cf. Malhi *et al.* 2006). This corresponds with the conclusion by Fauset *et al.* (2015), who stated that environmental conditions act as a much stronger constraint on the ability of a species to dominate a forest's metabolism (productivity and biomass storage) than on the presence of such a species alone.

However, in order to better understand how this influence of species composition on biomass is mediated through stand variables in tropical dry forest specifically, more research is needed. Within this research, no direct link between species composition and aboveground biomass was analysed, except for the presence of hyperdominant species. One way to do this more thoroughly though, could be by comparing AGB and species composition of Tucuman-Bolivian forests at different elevations, but with the same climatic (precipitation) conditions. If such a sampling design results in different aboveground biomass values, these can be attributed to differences in elevation. This, based on the conclusions above, should be related to different species compositions as well. The effect of elevation on stand variables, species composition and final AGB value can thus be studied, independently of a differential drought stress effect. However, this is only possible if sites with such characteristics can be found. Within this research, this was not possible, as the sites studied here each had different climatic conditions.

Overall though, based on this research, it can be concluded that both the biomass and species composition in the ANMI RG-VC show large variations at a landscape-scale. This further emphasizes the point made earlier that representativeness using multiple smaller plots is much required for landscape-scale extrapolation using remote sensing forest cover data.

5. Conclusion

In this research, it was tried to answer the following main research question: *What is the influence of environmental factors on regional AGB estimates for avoided deforestation projects in the tropical dry forests of the Río Grande - Valles Cruceños Natural Area of Integrated Management, and how is this related to forest structure and species composition?* By answering this, this research hoped to contribute to Fundación Natura Bolivia's efforts to assess avoided deforestation projects in terms of prevented carbon emissions. This could help for the future application of Fundación Natura Bolivia's Reciprocal Environmental Agreement schemes towards possible REDD+ projects.

In order to achieve this goal, three aspects of this question were analysed through three sub questions. The first sub question was: *What are the effects of different methodological choices on estimated AGB values?* It was found that the methodological choice for estimating tree height by eye or via allometric estimation causes the biggest difference in overall AGB estimates (40-45 per cent). The choice between 5 basic allometric equations causes a 20-35 per cent difference in final AGB estimates. On a landscape-scale this causes an overall difference of 35 per cent in AGB, based on the difference between Eq. 2 and 6. It was found that the second allometric equation under study, the tropical dry forest equation by Brown (1997), produces the most reliable AGB estimate for the ANMI RG-VC area, in the simplest way. For this reason, it is recommended to use this equation in any further analysis of biomass stocks in this region, unless a region-specific allometric equation becomes available.

The effect of plot size on AGB estimates, namely the choice between 0.1 and 1 ha plot size in TB forest, seemed limited. The former plot size resulted in a 10 per cent lower AGB estimate, on average, although this effect was found to be insignificant. This difference is smaller than the local spatial variation between plots at site level (10-20 per cent). For this reason, it is recommend to use multiple small forest plots of 0.1 ha (20x50m) for any further forest inventory in the ANMI RG-VC area. Each plot should then be representative of a single homogenous forest or vegetation type. For this reason, these plots are best spread out over a larger area than was the case in this study (which based its sampling design on 8 strata only, with 3 plots clustered together at each site). In this way, more of the landscape heterogeneity in the ANMI RG-VC can be captured by the sampling design, which reduces the landscape representation error, while the plot size error does not increase significantly. However, more research is still need on the effect of this smaller plot size for sparser vegetation types such as in IAD or CS forest.

The choice between two lower measurement limits, namely at DBH \geq 5 or \geq 10 cm, resulted in no difference at all. For this reason, it is recommended that the lower measurement limit for TB and CS forest is best set at 10 cm DBH, while 5 cm is best chosen for IAD forest. In this way, most of the tree biomass is taken into account, in the most efficient way.

The second sub question was: *What is the regional variation in estimated AGB, forest structure and species composition across the Río Grande - Valles Cruceños Natural Area of Integrated Management?* It was found that there is considerable spatial variation across the region, specifically in aboveground biomass, basal area, stem density and species composition. At the ecoregion level, a significant difference in AGB was found between Tucuman-Bolivian and Inter-Andean Dry forest (201 and 78

t/ha), while Chaco Serrano was found to store 178 t/ha of biomass. Within TB forest, no significant difference was found between evergreen and deciduous forest cover, as classified by RapidEye data. For this reason, the distinction in forest class types, as made in this research, is seen as ineffective. Hence it is recommended for future research to only differentiate between ecoregions and/or ecosystems, and not between cover classes (evergreen or deciduous forest). The RS remote sensing data is then only applied for calculations of total forested area.

At an ecosystem level, significant differences in AGB were found between the 8 main ecosystem types. However, for some types, only 1 forest plot was available due to fact that the sampling setup was designed at ecoregion level. For this reason, if future forest inventory plots are installed in the ANMI RG-VC, it is recommended to locate these within these under-sampled ecosystems, in order to obtain a better landscape coverage.

At regional scale, the ecoregion approach resulted in an estimated 108 Mt of biomass for the entire ANMI RG-VC, while the ecosystem approach resulted in a value of 101 Mt aboveground biomass. These approaches thus seem relatively comparable. On average this is 179 and 166 t/ha of forested area, respectively. This data can be applied by Fundación Natura Bolivia in analysing the effectiveness of its Reciprocal Environmental Agreements towards avoided carbon emissions.

However, in order to more precisely model the spatial distribution of AGB, the third sub question was: *Which stand or environmental factors contribute most to regional spatial variation in AGB and species composition, and how can these be used for mapping regional AGB values?* It was found that the year-round water limitation, in the form of the climatic water deficit, is the strongest predictive variable for AGB in Tucuman-Bolivian forest. However, difference between the three main ecoregions were found to be caused most strongly by drought stress extremities. This confirmed the hypothesis that precipitation is a stronger predictor than elevation for total biomass. Elevation was found to have no effect on AGB.

When AGB within the TB ecoregion was modelled based on these predictor variables, an average value of 183 t/ha was found, which is slightly lower than the TB plot average of 201 t/ha. However, the differences within the TB ecoregion become much larger when modelled according to these predictor variables.

In terms of species composition, though, elevation was found to have a strong predictive effect. Through the temperature gradient, elevation strongly affects species composition in TB forest. Species composition between the three ecoregions, however, turns out to be again related to drought stress.

Species composition was found to be very distinct for each different site, with generally 3-5 locationspecific hyperdominant trees present. The presence of these hyperdominant species thus seems to be related to the elevation effect within TB forest, and the drought stress effect between TB, IAD and CS ecoregions. Overall biomass, however, turned out to be only related to precipitation and drought effects. Thus, it was concluded that there is a differential environmental effect on aboveground biomass and species composition. Stem density and basal area were found to mediate the effect of species composition on biomass. It was concluded that more research is needed to better understand this link between species composition and biomass in the tropical dry forests of Bolivia.

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Appendix

Class Type	Class Name	# of plots	Eq. 1	Eq. 2	Eq. 3	Eq. 4	Eq. 5	Eq. 6	Eq. 7
Site	Pampa Negra	3	101,8	79,4	109,4	55,6	52,2	106,0	103,1
Site	Molleaguada	3	92,1	76,9	125,8	69,7	67,2	128,4	129,5
Site	Laja Toco	3	197,2	178,4	269,1	222,0	237,6	290,0	317,7
Site	Algodonales	3	160,3	144,6	176,2	132,5	130,3	185,1	187,9
Site	Тосоратра	3	177,3	165,1	228,0	172,2	170,6	233,5	238,3
Site	Bicoquin	2	378,8	349,7	470,9	424,6	459,5	512,2	559,3
Site	Chiriguanañan	3	201,8	191,9	240,7	165,2	159,2	257,1	256,5
Site	Salsipuedes Grande	1	223,3	210,1	204,7	139,4	133,2	230,8	229,8
Ecoregion	Tucuman-Bolivian forest	12	216,6	201,2	256,7	199,9	202,7	273,5	283,0
Ecoregion	Inter-Andean Dry forest	6	97,0	78,1	117,6	62,7	59,7	117,2	116,3
Ecoregion	Chaco Serrano	3	197,2	178,4	269,1	222,0	237,6	290,0	317,7
Ecosystem	CES409.213	1	79,6	67,8	107,0	66,2	60,8	104,3	98,9
Ecosystem	CES409.219	1	223,3	210,1	204,7	139,4	133,2	230,8	229,8
Ecosystem	CES409.211	5	100,4	80,2	119,7	62,0	59,5	119,7	119,8
Ecosystem	CES406.238	3	197,2	178,4	269,1	222,0	237,6	290,0	317,7
Ecosystem	CES409.206	3	160,3	144,6	176,2	132,5	130,3	185,1	187,9
Ecosystem	CES409.207	4	226,3	210,2	286,8	221,5	224,9	294,8	306,4
Ecosystem	CES409.197	3	201,8	191,9	240,7	165,2	159,2	257,1	256,5
Ecosystem	CES409.205	1	384,3	353,9	478,5	480,0	531,3	545,8	607,7

Average aboveground biomass values (tonnes/ha) per site location, ecoregion or ecosystem.