**Disentangling the mechanisms and uncovering the scale
of increasing liana size and abundance in neotropical forests**

By

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Preface

Governments, policymakers, and businesses are now asking ecologists for guidance as nations worldwide work to combat the causes and consequences of global anthropogenic change, especially those of climate change. Not only have ecologists become responsible for providing sound data on the effects of climate change, but are also being called on to provide reliable predictions. Ecologists, and scientists in general, increasingly need to balance their commitment to rigorous independent investigation of their study systems with the need to actively advocate for the conservation of those same systems. However, there is no inherent trade-off between these two activities. We as scientists are trained to be independent-minded, able to assess and draw conclusions based on the merits of the evidence at hand. Given the enormous challenges of global climate change and the threat it poses to our field, my generation of ecologists must aggressively use the expertise and understanding of natural systems we posses to educate those responsible for making decisions affecting the future of our planet. Only then can we truly say our work has made a lasting difference.

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Humans are altering tropical ecosystems and their biotic and biogeochemical processes with unprecedented scale and severity. The increasing size and abundance of tropical lianas (woody climbing plants) relative to trees may be the result of global anthropogenic change, and may further alter forest function. Yet the mechanisms responsible for this reported phenomenon are unclear, and the scale at which it occurs has been unexamined. In this dissertation, I use a combination of empirical experimentation, ground-based forest censuses, and advanced airborne remote sensing imagery and analysis to investigate the question of why lianas are increasing and at what spatial scale. First, I tested the hypothesis that elevated CO_2 gives species of lianas a growth advantage relative to trees, especially during periods of seasonal drought. In the first experiments to directly compare the relative response of tropical liana and tree species to elevated CO_2, I found no significant differences between the two growth forms. Both lianas and trees responded equally well to elevated CO_2, even when soil water was limited by seasonal drought. Second, I extended the tests of liana-tree response to CO_2 to include the effects of soil

nutrient availability. No interactions between elevated CO_2 and either soil nitrogen or phosphorus availability were found for lianas. Instead, changes to soil nutrient availability or CO_2 alone had strong and significant effects on lianas. Finally, I used data collected from my field censuses to train machine learning algorithms to detect severe liana coverage in tree canopies using high-resolution hyperspectral imagery. This method proved to be very accurate at distinguishing severe liana cover from liana-free cover in tree canopies, and quantified severe liana infestation as 11.9%-18.0% of the total canopy cover over a 600-ha tropical forest. The results of the experiments and the development of landscape-scale liana detection methods are key steps toward a full understanding of the mechanisms and scope of the liana increase.

Chapter I

Introduction

Forest dynamics, or the changes in community composition over time, are fundamental to the study of tropical forest community ecology. For years many ecologists assumed tropical forests eventually attained a state of 'dynamic equilibrium,' where tree growth and death rates are equal and composition (abundance and diversity) of species within a community remained stable (Whitmore, 1978). This assumption has been challenged recently (but as early as Connell (1978)), with studies revealing that biomass, productivity, mortality, recruitment, and community composition of neotropical forests are changing over time (Phillips *et al.*, 1998; Baker *et al.*, 2004; Laurance *et al.*, 2004; Phillips *et al.*, 2004). These pervasive changes have been attributed to global anthropogenic alterations of the biosphere and atmosphere. The unprecedented scale and severity on which humans are altering tropical ecosystems and their biotic and biogeochemical processes are of global consequence: tropical forests alone account for 60% of total terrestrial carbon uptake from the atmosphere (Pan et *al.*, 2011).

One particular concern is the reported increase in the size and abundance of lianas (woody climbing plants). In tropical forest ecosystems, trees and lianas represent the two dominant plant growth forms. In tropical and temperate forest surveys that include lianas, climbers comprise roughly one-quarter of the woody species richness (Table 1.1). Liana abundance within these forests, however, is more variable, ranging anywhere from 4 to 45% of woody stems in forests. In a study on Barro Colorado Island, Panama, Schnitzer & Carson

(2001) found lianas to represent almost 30% of the species richness in the seedling layer (with trees comprising 55%).

Across tropical forests lianas are consistently observed climbing in at least 50% of surveyed trees (Clark & Clark, 1990; Gentry, 1991; Campbell & Newbery, 1993; Ingwell *et al.*, 2010; van der Heijden *et al.*, 2010; Campanello *et al.*, 2012; Chapter 4) The close association between lianas and trees is a consequence of the liana growth strategy, whereby they depend on trees and other growth forms (including other lianas) for structural support (Putz & Mooney, 1991). The interactions between lianas and trees will be essential to understanding how future forest richness and composition may respond to anthropogenic global change.

Liana-Tree Interactions

The growth strategy, morphology, and physiology of lianas differ greatly from that of trees. Lianas invest few resources in mechanical support and instead rely on trees and other structures for support (Putz, 1984a). This shifts the available resource allocation to favor growth and reproduction. As a result, lianas have a high leaf area to stem mass ratio resulting in larger photosynthetic biomass than trees (Schnitzer & Bongers, 2002; Zhu & Cao, 2010; Paul & Yavitt, 2011). In addition, vertical growth rates of lianas outpace other woody life forms (Schnitzer, 2005). Rapid growth and the ability to follow virtually any structural support pathway (within the constraints of their specific climbing mechanism) confers a greater flexibility among lianas than trees in seeking out new sources or higher levels of light. The well-developed root system of lianas, combined with large vessel elements, allows lianas to efficiently and rapidly absorb and transport water and nutrients (Ewers *et al.*, 1991; Schnitzer, 2005; Foster & Brooks, 2005; Domingues *et al.*, 2007; Cai *et al.*, 2009).

Forest managers have long known that lianas negatively impact tree growth and regeneration (Putz, 1984a). Lianas have a disproportionately large negative effect on tree biomass accumulation by reducing tree diameter increment (Lowe & Walker, 1977; Whigham, 1984; Clark & Clark, 1990; Grauel & Putz, 2004; van der Heijden & Phillips, 2009; Schnitzer *et al.*, unpublished data), leaf productivity (Dillenburg *et al.*, 1993b; Perez-Salicrup, 2001; Toledo-Aceves & Swaine, 2008), sap flow velocity (Tobin *et al.*, 2012; Alvarez-Cansino *et al.*, unpublished data), and stem height (Perez-Salicrup, 2001). Lianas also decrease forest carbon accumulation and long-term storage (Duran & Gianoli, 2013) through reduced tree fecundity (Stevens, 1987; Kainer *et al.*, 2006; Nabe-Nielsen *et al.*, 2009), increased tree mortality (Putz, 1984b; Phillips *et al.*, 2002; Garrido-Perez *et al.*, 2008; Ingwell *et al.*, 2010; Schnitzer *et al.*, unpublished data), and suppressed tree regeneration (Toledo-Aceves & Swaine, 2008; Schnitzer & Carson, 2010). These effects of lianas are achieved not only through classic competitive mechanisms, but also through unconventional interactions specific to lianas.

Intense aboveground interactions between lianas and trees results in decreased tree growth. Lianas use tree stems as structural support in order to reach the canopy where light levels are the highest. Once in the canopy, lianas spread outward and in some cases form a dense layer of vegetation, shading out the leaves of trees below (Dillenburg *et al.*, 1993b; Avalos *et al.*, 1999). In addition to competition for light in the canopy, lianas grow rapidly into light gaps that open as a result of a disturbance (Schnitzer *et al.*, 2000). Their ability to grow into and dominate forest gaps allows them to outcompete trees for space. Lianas have been found in such high densities in treefall gaps in tropical forests that they mechanically prevent trees from growing upward (Schnitzer *et al.*, 2000).

While belowground competition was for some time ignored as a substantial component of liana-tree interactions, recent studies have found it may be at least as important as aboveground competition. Experiments restricting liana-tree interactions to only belowground competition have found that tree growth rates and biomass were reduced (Dillenburg *et al.*, 1993a; 1993b; 1995; Barker & Perez-Salicrup, 2000; Schnitzer *et al.*, 2005; Chen *et al.*, 2008). In a study testing the effect of light on the relative importance of above- and belowground competition, Chen *et al.* (2008) found that in high light conditions, belowground competition between lianas and trees significantly and negatively affected tree seedling development.

Studies of belowground competition between lianas and trees not only investigated biomass and growth rates, but also the cause of the growth decrease. Dillenburg *et al.* (1993a) found that trees had lower leaf nitrogen concentration in belowground competition treatments than in either aboveground only or above- and belowground competition, suggesting that soil nitrogen availability was responsible for the competitive interaction. However, results from studies of competition for soil water are mixed. Soil water availability was significantly reduced when measured in trees with lianas than in trees whose lianas were experimentally cut (Perez-Salicrup & Barker, 2000). In a separate liana removal experiment, Barker & Perez-Salicrup (2000) found that liana competition did not reduce tree water status even after a prolonged dry period.

Lianas also indirectly compete with trees through unconventional means. As multiple individual lianas climb a single tree, or as one liana accumulates biomass in a tree canopy, the added weight can exert torque on the stem and roots increasing the likelihood of tree mortality or reduced growth (Putz 1984a). If a liana uses twining as its climbing mechanism it may constrict a tree's phloem as it tightens around the bole of the tree, reducing translocation of nutrients

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(Hegarty 1991). When a liana reaches a tree canopy it can grow into neighboring tree crowns thereby binding the trees together. In the tropics a liana, on average, climbs in more than one tree (Putz 1984b), increasing the chance that a single tree falling will bring down multiple trees at the same time. In a survey of treefall events, Putz (1984b) found that many trees were brought down along with the causal tree as a consequence of lianas linking them. The number of trees that fell from a hurricane event was lower in an experimental plot where lianas were removed from the trees than the unmanipulated controls (Garrido-Perez *et al*. 2008). Yet, it is rare that a liana will die when brought down in a treefall. In fact, 90% of liana stems survive such an event (Putz 1984b). Each of these mechanisms serves to give lianas an added advantage when competing with trees for limited resources.

Increasing Liana Size and Abundance

Due to their importance to forest structure and function, lianas have attracted increasing interest over the past two decades (Gerwing *et al.*, 2006). A result of the increasing study of lianas has been the discovery that their size and abundance are increasing. In a synthesis of studies from multiple neotropical plots, stem density and basal area of large lianas was documented to have increased by 4.3% per year over the last two decades of the 20th century (Phillips *et al*. 2002), representing nearly a doubling in proportional liana basal area. More recent studies have reported annual increases in liana stem abundance ranging from 0.23% to 7.8%, while in the same study areas trees either underwent smaller annual increases or have declined in stem abundance (Phillips *et al.*, 2002; Chave *et al.*, 2008; Schnitzer *et al.*, 2012; Yorke *et al.*, 2013). These same studies found increases in liana biomass or basal area ranging from 0.6% to 4.6% annually over the same time period. And again, over the same period, only a 0.34% per year increase in tropical tree biomass or basal area was reported.

Proxy evidence for the increasing size and abundance of lianas also exist. Benítez-Malvido & Martinez-Ramos (2003) found a 500% increase in liana seedling recruitment over a six and a half year period (1993-1999) in a Brazilian lowland moist forest. Ingwell *et al*. (2011) documented a 57% increase in the proliferation of lianas in tropical tree canopies since 1980. Similarly, in tropical Panamanian forests liana leaf litter production increased by 55% over 17 years (Wright *et al.*, 2004). Furthermore, Wright & Calderon (2006) calculated a 4.1% and 1.8% per year increase from 1987 to 2003 in flower production of lianas and trees, respectively. These trends have been implicated in the recent increase of neotropical tree mortality, with lianas being associated with a 40% to 100% increased risk of tree mortality (Phillips *et al*. 2002, Ingwell *et al*. 2011).

The trends are not restricted to the tropics. Allen *et al.* (2007) found that liana importance (as measured by density, stem proportion, and basal area) has increased across a southeastern U.S. temperate forest. However, Londre & Schnitzer (2006) did not find any increase in the abundance of lianas in a northern temperate forest, suggesting that the liana increase is limited to the tropics and subtropics.

Proposed mechanisms to explain the trend of increasing liana size and abundance (reviewed by Schnitzer & Bongers, 2011) include increasing rates of natural and anthropogenic disturbances, increasing length and severity of dry seasons, and increasing atmospheric carbon dioxide (CO_2). Elevated CO_2 is often invoked as a main cause of increasing lianas, with measurements of tropical liana species exposed to elevated CO_2 showing increases in biomass, height, leaf area, and root mass compared to ambient CO_2 (Condon *et al.*, 1992; Korner & Arnone, 1992; Granados & Korner, 2002). These latter three studies are the only known experiments to have exposed tropical lianas to elevated CO_2 and included only 8 species.

Despite the known negative consequences of increasing lianas, few studies have investigated the underlying causes or examined the scale of the increase in tropical liana abundance and size. In this dissertation I investigate why lianas are increasing and on what spatial scale. I describe a series of experiments designed to investigate one of the main proposed mechanisms for the liana increase: increasing atmospheric CO_2 (Chapter 2). Since tropical forest soils are often assumed to be nutrient deficient, a subset of these experiments manipulated nutrient availability to test interactions with elevated CO_2 (Chapter 3). Even if we were to fully understand the underlying mechanisms that could give lianas a further competitive edge over trees, an understanding of the spatial scale on which liana infestations can occur is needed to assess the magnitude of the problem (Chapter 4). Each of these chapters is introduced in more detail below.

Chapter 2: No evidence that elevated CO_2 gives tropical lianas an advantage over tropical trees. In this chapter I describe two experiments I conducted in Panama testing the hypothesis that elevated CO_2 confers a growth advantage on tropical lianas relative to trees. While prior studies found tropical lianas respond with increased growth and biomass to elevated CO_2, no study had yet simultaneously tested the response of lianas and trees grown under elevated CO_2. Without comparing the relative response to CO_2 of both growth forms in the same experiment, we cannot demonstrate whether lianas respond more to atmospheric CO_2 increases than trees. The two studies presented in this chapter explicitly tested the response to elevated CO_2 of seedlings of 11 tropical liana and 10 tropical tree species growing together in the ground, and whether seasonal drought altered the response of either growth form.

Chapter 3: The relative growth response of tropical lianas to elevated CO_2 does not depend on soil nutrient availability. Here I describe two multifactorial experiments that tested

whether changes in soil nutrient availability produce differences in the relative response of lianas and trees to elevated CO_2. While phosphorus availability has long been recognized as a key constraint of lowland tropical forest productivity, recent evidence suggests that nitrogen may also be in limiting supply (Wright *et al.*, 2011). As global biogeochemical cycles of carbon, nitrogen, and phosphorus continue to change, increasing atmospheric CO_2, higher rates of nitrogen deposition, and decreasing soil phosphorus levels may interact to give tropical lianas an advantage over trees. In one experiment, seedlings of two liana and two tree species were grown in pots with low and high soil nitrogen. In a second experiment, seedlings of three liana species were grown in pots with low and high soil phosphorus. Both experiments measured species response to elevated CO_2.

Chapter 4: Mapping liana canopy cover across tropical forest landscapes using high-resolution imaging spectroscopy. This chapter presents the results of a new approach to mapping the tree canopy coverage of lianas at the landscape scale in contiguous tropical forests. The historical exclusion of lianas from many forest censuses has resulted in a severe lack of data (both spatially and temporally) from which we can assess the scale and impact of increasing liana size and abundance relative to trees. Remotely sensed (satellite and airborne) data of tropical forests can potentially provide these data at far larger spatial and temporal scales than plot-based censuses. The contrasting foliar chemical and structural properties of lianas and trees are reflected in different spectral reflectance patterns that may allow the two growth forms to be distinguished at the sub-canopy scale. I combined advanced airborne imaging spectroscopy with a ground-based census and machine learning classification techniques to investigate the accuracy of detecting individual tree crowns with severe liana cover (>80%) from those with no lianas.

This approach was applied to a nearly 600 ha intact tropical forest to quantify and examine the distribution of high liana coverage at the landscape scale.

My concluding section frames the results of my work in the broader context of understanding the effects of global change on tropical forests.

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Location	Richness (%)	Abundance (%)	Reference
Ecuador	26	12	Gentry and Dodson (1987)
Malaysia	24	26	Appanah et al. (1993)
Borneo	14	10	Appanah et al. (1993)
Panama	NA	22	DeWalt et al. (2000)
India	33	45	Muthuramkumar and Parthasarathy (2001)
Nigeria	48	39	Muoghalu and Okeesan (2005)
NE North America	13	NA	Quigley and Plantt (1996)
	22	19	R Burnham (unpublished data)
SE North America	29	NA	Quigley and Plantt (1996)

Table 1.1. Liana species richness and abundance (as a percent of total woody plants) from selected plots across the world. Note: Averages of several sites are reported in places where multiple plots exist.

Chapter II

No evidence that elevated CO_2 gives tropical lianas an advantage over tropical trees[*]

Abstract

Increases in the size and abundance of lianas relative to trees in neotropical forests have been reported in recent decades. As a result, forest dynamics and carbon balance may be altered through the suppression of tree growth and increases in tree mortality by lianas. Increasing atmospheric CO_2 is hypothesized as one mechanism causing the reported tropical liana increase, yet no study has directly compared the relative response of tropical lianas and trees to elevated CO_2. For the first time, we explicitly tested whether tropical lianas had a relatively larger response to elevated CO_2 compared to tropical trees, and whether seasonal drought alters the response of either growth form. In two experiments conducted in central Panama, one spanning both wet and dry seasons and one conducted only during the dry season, we grew locally abundant liana (n=11) and tree (n=10) species in open-top growth chambers maintained at ambient or twice-ambient CO_2 levels. Seedlings of eight individuals (four lianas, four trees) were grown in the ground in each chamber for at least three months during each season. We found that both lianas and trees had a significant and positive response to elevated CO_2 (in biomass, leaf

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area, leaf mass per area, and photosynthesis), but that the relative response to elevated CO_2 was

not significantly greater for lianas than trees in all response variables measured. The lack of

differences in the relative response between growth forms leads us to conclude that elevated CO_2

is unlikely the main mechanism underlying the reported increases in liana abundance and size

across the neotropics.

Introduction

      Lianas (woody vines) are increasing in size and abundance relative to trees throughout

neotropical forests (Schnitzer & Bongers, 2011; Schnitzer *et al.*, 2012; Yorke *et al.*, 2013).

Reported annual increases in liana abundance range from 0.23% to 7.8% over recent decades,

whereas trees either underwent smaller annual increases or have declined in abundance in the

same study areas (Phillips *et al*. 2002; Chave *et al.*, 2008; Schnitzer *et al.*, 2012). Liana seedling

recruitment, reproduction, and leaf productivity have also increased relative to trees (Wright *et

al.*, 2004; Wright & Calderon, 2006; Benitez-Malvido & Martinez-Ramos, 2008).

      The reported liana increases have broad implications for the global carbon cycle because

tropical forests account for the single largest terrestrial share (60%) of annual global carbon

dioxide uptake (Pan *et al.*, 2011). The negative effect that lianas exert on tree growth,

reproduction, and lifespan, combined with their very low contribution to forest biomass, suggest

a future in which neotropical forests will absorb and store less atmospheric carbon dioxide

annually.

      Lianas commonly comprise a large proportion of the woody species and stem number in

tropical forests (Schnitzer *et al.*, 2012); however, lianas constitute a small proportion of total

tropical forest biomass (Putz, 1983; Gerwing & Farias, 2000; DeWalt & Chave, 2004; Letcher &

Chazdon, 2009). Nevertheless, lianas have a disproportionately large negative effect on tree

biomass accumulation by reducing tree diameter increment (Lowe & Walker, 1977; Whigham, 1984; Clark & Clark, 1990; Grauel & Putz, 2004; van der Heijden & Phillips, 2009; Schnitzer *et al.*, unpublished data), leaf productivity (Dillenburg *et al.*, 1993; Perez-Salicrup *et al.*, 2001; Toledo-Aceves & Swaine, 2008), sap flow velocity (Tobin *et al.*, 2012; Alvarez-Cansino *et al.*, unpublished data), and stem height (Perez-Salicrup, 2001). Lianas also decrease forest carbon accumulation and long-term storage through reduced tree fecundity (Stevens, 1987; Kainer *et al.*, 2006; Nabe-Nielsen *et al.*, 2009), increased tree mortality (Putz, 1984; Phillips *et al.*, 2002; Garrido-Perez *et al.*, 2008; Ingwell *et al.*, 2010; Schnitzer *et al.*, in review), and suppressed tree regeneration (Toledo-Aceves & Swaine, 2008; Schnitzer & Carson, 2010). Depending on the level of infestation, lianas are associated with a 40-100% increase in tree mortality (Phillips *et al.*, 2002; Ingwell *et al.*, 2010).

The causes of increasing lianas have not been empirically determined, but the main putative mechanisms include: increased intensity of seasonal drought, higher rates of natural and anthropogenic disturbance, and increasing atmospheric CO_2 (Phillips *et al.*, 2002; Schnitzer & Bongers, 2011). Increasing atmospheric CO_2 is often invoked as a main cause of increasing lianas (e.g., Phillips *et al.*, 2002) because global atmospheric CO_2 levels have increased 41% since 1750 (IPCC, 2001), with well over half the increase occurring since 1960 (NOAA, 2013). Because lianas invest less in structural support, relying instead on trees for access to the high-light environment of forest canopies, their ratio of leaf area to stem or total plant biomass (LAR) is higher than in trees (Zhu & Cao, 2009; 2010; Paul & Yavitt, 2011). The high LAR of lianas may allow them to take advantage of increases in CO_2 levels to a greater extent than can trees (Schnitzer & Bongers, 2011). Lianas and trees have similar photosynthetic capacity per unit leaf area (Asner & Martin, 2012), therefore lianas should gain proportionally more carbon per unit of

plant mass due to their relatively greater leaf area. This additional carbon should give lianas an advantage over trees through greater growth and reproduction, leading to increasing liana density, biomass, and productivity relative to trees in tropical forests.

To date, the empirical evidence for increasing atmospheric CO_2 as the cause of increasing tropical lianas is derived from just three greenhouse studies of lianas grown in ambient and elevated CO_2 – none of which compared the response of lianas to trees. Granados & Korner (2002) found an increase in biomass for three tropical liana species grown under elevated CO_2, but did not find a consistent growth response for other traits. Condon *et al*. (1992) reported that two congeneric species of tropical lianas exposed to elevated CO_2 increased in total biomass, leaf area, and height compared to ambient CO_2. Korner & Arnone (1992) found neither an aboveground biomass response nor an increase in leaf area index, but did find increased root mass under elevated CO_2 for a model community that included two liana and three tree species. However, their reported results did not compare the responses between the two growth forms. While there is evidence that some tropical liana species respond to elevated CO_2, previous studies have not simultaneously and explicitly compared tropical lianas to trees, and consequently are unable to demonstrate that lianas respond more than trees to increased atmospheric CO_2. Furthermore, none of these studies were conducted in-ground in the tropics.

Lianas may have a further advantage over trees under elevated atmospheric CO_2 in forests that experience seasonal drought. Relative to trees, liana stems increase in abundance in neotropical forests as annual precipitation decreases (Schnitzer 2005). This dry season advantage may result from the inherently higher water use efficiency of lianas (Cai *et al.*, 2009) combined with the potential ability of lianas to access moisture from deeper soil strata than trees (Schnitzer 2005). Elevated CO_2 is known to increases the water use efficiency of plants by reducing

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stomatal conductance and increasing rates of photosynthesis (Battipaglia *et al.*, 2012; Cernusak *et al.*, 2013), thus allowing more carbon to be fixed per unit water lost through transpiration. Seasonal drought-adapted lianas should increase carbon fixation, and thus water use efficiency, proportionally more than trees under elevated CO_2 because water-stress or deciduousness may limit carbon gain in many trees during periods of seasonal drought (Schnitzer & Bongers, 2011). This hypothesis has not been empirically tested in tropical lianas and trees.

We tested the hypothesis that lianas respond more than trees to elevated atmospheric CO_2 using a large and phylogenetically diverse set of liana and tree species in common gardens in the tropics. We examined the growth of seedlings of eleven liana species and ten tree species grown in the ground within open-top chambers maintained at either ambient or elevated CO_2. We included seasonal drought as a factor and examined the response of both growth forms to elevated CO_2 over two studies: one conducted during the dry season only ("dry-only") and one conducted during both wet and dry seasons ("wet-dry"). We asked whether 1) elevated CO_2 differentially affects the growth of tropical lianas and trees and 2) seasonal drought alters the response of these growth forms to elevated CO_2. We hypothesized that: 1) lianas would show larger relative growth than trees under elevated CO_2 due to higher proportional investment in leaf area and photosynthesis than trees, and 2) lianas would increase their water use efficiency more than trees during seasonal drought in elevated CO_2, which would offset decreases in growth during the dry season to a greater extent for lianas than trees.

Materials and Methods

Site and Species

We conducted the study along a forest edge at the Smithsonian Tropical Research Institute's Experimental Plant Growth Facility in the Republic of Panama (Figure 2.1a). Over the past seven years the Smithsonian collected hourly readings of temperature, precipitation, and full-sun photosynthetically active radiation (PAR) at this site. During the wet season (May-December) the monthly average daytime temperature is 27.9 °C, average monthly precipitation is 244 mm, and average daily total PAR is 25.2 mol m^{-2}. During the dry season (January-April) the monthly average daytime temperature is 29.3 °C, average monthly precipitation is 44 mm, and average daily total PAR is 33.8 mol m^{-2}.

We constructed an array of 36 open top growth chambers measuring 1 m length x 1 m width x 2 m height, spaced approximately 1.5 m from each other, and wrapped with 90% shade cloth to reduce incoming sunlight and interior temperature. An air delivery system composed of three industrial blower fans attached to plastic plenums (4 m length x 1 m diameter) fed each chamber through 10 cm diameter flexible dryer ducting. Metal duct dampers controlled the ambient airflow rate through the ducting to exchange the air in each chamber once every two minutes (see Supplemental Methods for details). Half of the chambers received pure CO_2 regulated through manual flow meters to a level of 780 μmol mol^{-1}. An automated sampling system and infrared gas analyzer monitored levels of CO_2 in all elevated and two ambient chambers (see Supplemental Methods for details). Sensors inside and outside a subset of chambers monitored temperature, light, and soil volumetric water content (VWC) throughout each experiment (see Supplemental Methods for details). At the end of each experiment, and after the harvest, we extracted and homogenized four soil samples from the upper 5 cm of each chamber. We analyzed each homogenized sample for ammonium, nitrate, and total mineral element concentrations (see Supplemental Methods for details). We extracted, dried, and

weighed fine root material of non-experimental plants growing into the chamber soil from each of the homogenized samples.

We used eleven liana and ten tree species in the two separate experiments reported here (Table 2.1). We selected the species from among the most common species in central Panama (DeWalt *et al.*, 2000; Hubbell *et al.*, 2005; Schnitzer *et al.*, 2012) and across a range of life history strategies. The availability of fruits, seeds, and seedlings from Barro Colorado Nature Monument forests, and from local reforestation nurseries, also guided species selection. We used a phylogenetically diverse group of liana and tree species to apply the experimental results more broadly to neotropical terrestrial communities.

Experimental Design

We conducted two experiments: a three month "dry-only" experiment starting February 2011, and a seven month "wet-dry" season experiment starting September 2011. In both the dry-only and wet-dry season experiments, we transplanted newly germinated seedlings (with at least one fully-expanded true leaf) into the chambers and allowed them to establish for 30 days before starting the CO_2 treatment.

The dry-only CO_2 treatment began in late February 2011, one month after the end of the wet season that year, and ran for 90 days, until late May. Although the wet season normally starts in early May, the precipitation during the May portion of the experiment (98 mm) was 48% below the historical average, and soil VWC in the chambers did not change between April and May. In the dry-only experiment we used a randomized complete block design, in which eight species of lianas and eight species of trees were randomly assigned to one of eight subplots within a pair of chambers (block) with the restriction that four distinct liana and four distinct tree species be in each chamber (Figure 2.1b). Species-level replication was nine individuals per CO_2

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treatment, resulting in 72 individuals of each growth form per CO_2 treatment. Due to the young age of the seedlings and high temperatures during the dry-only experiment, we applied supplemental water to maintain daily soil moisture at 30% VWC. Average soil moisture in the chambers without supplemental water during the subsequent (2012) dry season was 30% VWC (Table S2.1).

The wet-dry season CO_2 treatment began in September 2011 and ran until the end of March 2012 (204 days). In this experiment, we used a balanced factorial design, with four species of lianas and four species of trees randomly assigned to the eight subplots within each chamber. Species-level replication was 18 individuals per CO_2 treatment, resulting in 72 individuals of each growth form per CO_2 treatment. We did not use supplemental watering during this experiment. To reduce soil nutrient heterogeneity within the chamber plots, we removed, homogenized, and returned the top 50 cm of soil from all plots. We added up to 5 cm of soil from a nearby site to each growth chamber plot to compensate for soil lost during this process and during the root excavation at the end of the previous experiment. To reduce growth of roots into the chamber soil from nearby trees, we dug, lined with plastic, and backfilled a 75 cm deep trench around the entire site.

Plant Measurements

At the beginning of each experiment, we harvested 12 to 20 extra seedlings per species not used in the experiment and measured the height of the apical bud above soil (cm), diameter at 5 cm height (mm), number of live leaves, leaf area (cm^2), and dry above- and belowground biomass (g). We used these data to estimate the biomass of the experimental seedlings allometrically at the start of the experiment (see Supplemental Methods). We used the initial

biomass estimates to calculate the relative growth rate (RGR) of the biomass of each plant during the experiment:

$$RGR = \frac{\ln(M_{final}) - \ln(M_{init})}{t} \tag{1}$$

where M_{init} is the allometrically estimated dry biomass of each plant at the start of the treatment, M_{final} is the measured dry biomass at harvest, and t is the number of days between the treatment start and plant harvest.

Every fifteen days during both experiments we measured the diameter, height, and live and dead leaf count for each plant. During the wet-dry season experiment, three weeks before the end of the wet season, we measured the length (cm) and width (cm) of every leaf and leaflet to calculate approximate leaf area. After the harvest we measured 50 to 100 leaves from each species for length, width, and fresh leaf area using a leaf area meter (LI-3100C, LI-COR, Nebraska, USA). We used these data with stem diameter, height, and number of live leaves to allometrically estimate the total biomass of each plant mid-way through the experiment (see Supplemental Methods).

One week prior to the end of each experiment, and three weeks prior to the end of the wet season in the wet-dry experiment, we collected gas exchange measurements from the newest fully-expanded leaf on all plants. A portable photosynthesis system (6400XT, LI-COR, Nebraska, USA) measured the maximum photosynthetic rate (μmol CO_2 m^2 s^{-1}), stomatal conductance (mol H_2O m^2 s^{-1}), and transpiration rate (mmol H_2O m^2 s^{-1}). Inside the leaf chamber of the photosynthesis system, we set light levels to 1000 μmol m^{-2} s^{-1} PAR and CO_2 concentration to the appropriate chamber target level (i.e., 390 μmol mol^{-1} or 780 μmol mol^{-1}). Maximum (i.e., light saturated) photosynthetic rate is a commonly used parameter in studies

evaluating changes in photosynthetic capacity in response to elevated CO_2 (see Curtis & Wang 1998).

At the end of each experiment, in addition to the final biweekly measurements, we harvested all plants above and below ground, and measured the dry biomass of leaves, stems, and roots. Total leaf production is defined here as the difference between the number of live leaves at the beginning and number at the end of the treatment, plus all dead leaves. Leaf loss is calculated as the total number of dead leaves regardless of the mechanism (e.g., abscission, herbivory, pathogen). We ground approximately 200 mg of dried leaf material for each plant to a powder and measured the ratio of carbon to nitrogen (C:N) by combustion and thermal conductivity on a Thermo Flash EA112 analyzer (CE Elantech, New Jersey, USA).

Data Processing and Analysis

We performed all data processing and analysis in the open-source statistical software program R (R Development Core Team, 2012). See Supplemental Methods for a description of the allometric estimations and processing of site abiotic data.

The plant response variables analyzed in each experiment are presented in Table 2.2. To test each response variable for categorical treatment main effects and interactions we fit linear mixed-effects models with restricted maximum likelihood (REML) estimation (Pinhero & Bates, 2000) in the R package 'lme4' (Bates *et al.*, 2012). CO_2 treatment (elevated and ambient), growth form (liana and tree), and their interaction are fixed effects in the model. We used fixed and random effects in the model because we wished to examine growth form differences while still accounting for species-level differences. To account for chamber-to-chamber variability we used environmental variables measured within the growth chambers as covariates in the model. These include total PAR, average soil moisture (VWC), standard deviation of CO_2 concentration,

soil ammonium and nitrate, and the fine root biomass of non-experimental species growing into

the chamber plots (see Supplemental Methods for a summary of each covariate). To make the

coefficients directly comparable we standardized all covariates by subtracting the mean and

dividing by two standard deviations (Gelman & Hill, 2007). Random effects were included for

chamber to account for any extra-treatment variation not captured by the covariates, and for

species to account for species variation not due to growth form and treatment. For i individuals

in the wet-dry season we used a linear mixed effects model of the form:

$$\text{Response}_i = \alpha_{CO_2(i),GF(i)} + \delta_{Covariates(i)} + \left(\beta_{Chamber(i)} + \gamma_{Species(i)} \right) + \varepsilon_i \tag{2}$$

where Response_i is one of the measured plant response variables (Table 2.2). Fixed effects

$\alpha_{CO_2(i),GF(i)}$ represent the set of regression coefficients for each treatment and their interaction,

and $\delta_{Covariates(i)}$ represent the environmental variables used as covariates. The crossed random

effects structure $\beta_{Chamber(i)}$ and $\gamma_{Species(i)}$ allow the regression intercepts to vary, and ε_i are the

residual model errors. For i individuals in the dry-only experiment we used a model of the form:

$$\text{Response}_i = \alpha_{CO_2(i),GF(i)} + \delta_{Covariates(i)} + \left(\beta_{Block(i)} + \gamma_{Species(i)} \right) + \varepsilon_i \tag{3}$$

where each term is the same as in (2) except the random effect $\beta_{Block(i)}$ is used to allow intercepts

to vary by block rather than chamber to reflect the block design of this experiment.

We tested one alternate random effects structure for the models with only $\gamma_{Species(i)}$ as the

random intercept. We chose the optimal random effects structure for each response variable

using likelihood ratio tests in a simplified model containing only covariates. When chamber-to-

chamber variation was small to nonexistent, this alternate "species-only" random effects

structure was selected.

To generate p-values for each model coefficient, we used code adapted from Moore

(2010) that iteratively fits reduced fixed effects models and compares them to the full fixed

effects model using a likelihood ratio test. These models are all fit using maximum likelihood estimation instead of REML because REML estimates are not comparable among models with different fixed effects structures (Pinhero & Bates, 2000). When the interaction or a main effect term was not significant, the term(s) were removed and the model refit using the same procedure as above.

We used the R package 'lsmeans' (Lenth, 2013) to calculate the least squares means for each level of CO_2 and growth form in the interaction model. From these data we calculated the mean effect size (i.e., log response ratio) and the 95% confidence interval of the effect size separately for the liana and tree response to elevated CO_2 following the method of Hedges *et al.* (1999).

Results

Among the 19 growth and physiological response variables analyzed in the experiments, there were no significant differences in the relative effect of CO_2 on lianas versus trees (Table 2.3). While lianas tended to have a larger relative response to elevated CO_2, the lack of a significant interaction between CO_2 and growth form can be clearly seen across all response variables (Figures 2.2 and 2.3). There were very few variables where the two growth forms differed significantly, even when pooling the data across CO_2 treatments (Table 2.3). The substantial intra- and interspecific variation in the experiment shows that common species of these two growth forms do not respond in a clear and predictable manner to elevated CO_2. Full results from the linear mixed model estimations are presented in Tables 2.4 and 2.5.

While no differences between growth forms were found, a number of response variables had a significant and large CO_2 fertilization effect when pooled across growth form (Table 2.3) – evidence that validates the design of our experimental array and CO_2 treatment procedures.

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These CO_2 main effects are briefly discussed below for each experiment, and then for the wet and dry halves of the wet-dry season experiment.

Dry-only and wet-dry season experiments

In the dry-only experiment, four response variables showed a significant response to elevated CO_2 when growth forms are pooled (Table 2.3). Stem diameter significantly increased 24.7%, even though this is only an absolute change of < 1 mm. Root mass significantly increased 37.4%, while the aboveground biomass components (leaf and stem mass) did not show a significant increase in response to elevated CO_2. Leaf mass per area, a measure of a plant's investment in light interception (Poorter *et al.*, 2009), significantly increased 5.4%. The maximum photosynthetic rate significant increase of 37.3%, combined with no significant change in stomatal conductance or transpiration, meant an increase in water use efficiency for both lianas and trees.

The wet-dry season experiment, which ran for twice as long as the dry-only experiment but included half the number of species, also resulted in several significant differences between elevated and ambient CO_2. Significant leaf-level responses to elevated CO_2 included a 31.5% increase in leaf area and a 49.0% increase in leaf mass. Stem biomass significantly increased by 84.6%, the largest percentage increase of all the variables. Total plant biomass increased significantly over the study period, with an increase of 64.8% in response to elevated CO_2. Response of plant gas exchange to elevated CO_2 in this experiment is discussed below in the context of each half of the experiment because of the strong influence of seasonality.

Wet half and dry half growth and biomass response

During the wet half of the wet-dry season experiment, none of the growth or biomass response variables showed a significant response to elevated CO_2. However, in the dry half leaf

area showed a significant increase in response to elevated CO_2 of 37.2%. Total biomass change during the dry half increased significantly between ambient and elevated CO_2 by 69.8%, with RGR significantly increasing 19.0% in response to elevated CO_2.

Wet half and dry half physiological response

Elevated CO_2 caused significant increases in maximum photosynthetic rate in both the wet and dry halves of the wet-dry season experiment, with a 36.0% increase in the wet half and a 48.2% increase in the dry half. In the wet half, there was a positive but non-significant response to CO_2 for stomatal conductance and transpiration, whereas in the dry season stomatal conductance significantly decreased 28.9% and transpiration decreased 19.5%. These results indicate that water use efficiency increased in both seasons but did not differ between lianas and trees.

Random effects of chamber and species

Examining the random effects structures selected by the likelihood ratio test for the analysis of each response variable, we find that the crossed random effects structure (chamber and species) was selected for just over half the variables in the dry-only and the wet and dry halves of the wet-dry experiment. The crossed random effects structure was only selected in 4 of 14 response variables in the wet-dry experiment analysis. In the cases where the crossed random effects structure was selected, there was sufficient between chamber extra-treatment variation to include chamber as a random effect in addition to species. When the "species-only" random effects structure was selected, there was either little to no between chamber extra-treatment variability or the environmental covariates measured throughout the experiment sufficiently explained the chamber-to-chamber variability.

Discussion

We did not find strong empirical support for the hypothesis that lianas respond more than trees to elevated CO_2 in the first two experiments conducted in the tropics to compare growth forms. Based on the lack of any significant, stronger relative responses by lianas to elevated CO_2 across the variables measured, we find it unlikely that increasing atmospheric CO_2 is the main mechanism underlying the reported increase in neotropical liana size and abundance.

As the locus of CO_2 absorption and carbon fixation, leaf-level variables should show a strong response if the liana growth form had an inherent advantage over trees under elevated CO_2. However, we did not find support for the hypothesis that lianas invest more than trees in photosynthetic tissue under elevated CO_2. For all leaf variables measured in each experiment, lianas and trees invested a similar amount of resources, or did not show a significant response, when exposed to elevated CO_2. We found a moderate increase in leaf area and leaf biomass in response to elevated CO_2 during the wet-dry experiment, but this did not differ between lianas and trees. In the dry-only experiment, both lianas and trees invested similarly in the leaf-level cost of light interception (leaf mass per area).

There was not a significantly greater increase in liana biomass or height than trees in response to elevated CO_2. We therefore find no support for the hypothesis that high leaf area ratio (LAR) strategy of lianas necessarily confers an advantage under elevated CO_2. This hypothesis has been suggested as one of the underlying mechanisms explaining the reported increase in lianas (Mohan *et al.*, 2006; Körner, 2009; Schnitzer & Bongers, 2011). In fact, lianas and trees either had a very similar LAR, or trees had significantly larger LAR than lianas, at the end of each experiment.

We were surprised that lianas did not show a larger relative physiological response to elevated CO_2 during seasonal drought than trees, given their higher water use efficiency at ambient CO_2 levels, wider vessel elements, and potentially deeper root systems (Schnitzer, 2005; Foster & Brooks, 2005; Domingues *et al.*, 2007; Cai *et al.*, 2009). Many lianas retain their leaves and are able to increase their relative growth during the dry season (Putz & Windsor, 1987; Schnitzer, 2005), whereas many trees are deciduous or reduce their photosynthetic activity (Condit *et al.*, 2000; Schnitzer, 2005; Cai *et al.*, 2009). We anticipated lianas to take advantage of increased water use efficiency that elevated CO_2 imparts on plants (Battipaglia *et al.*, 2012).

However in the first reported gas exchange measurements conducted on tropical lianas under elevated CO_2, we found no significant differences in the relative increase in maximum photosynthetic rate between lianas and trees in either the wet or dry seasons. Similarly, we did not find any significant differences in the relative decrease in stomatal conductance and transpiration shown by lianas and trees. In both studies we found increases in water use efficiency, but there was no difference between lianas and trees. The lack of physiological differences between lianas and trees in response to CO_2 is reflected in their similar growth response. This runs contrary to our hypothesis that a greater increase in the water use efficiency of lianas compared to trees would offset dry season-induced growth reductions in lianas.

Our results are consistent with previous studies of lianas grown under elevated and ambient CO_2. All three prior studies found increases in either aboveground or belowground liana biomass in response to elevated CO_2. In two of the studies no other significant positive responses at the growth-form level were detected (Korner & Arnone, 1992; Granados & Korner, 2002), while Condon *et al*. (1992) found lianas also increased significantly in leaf area and height. We find similar results for the response of plant biomass and leaf area to elevated CO_2, but we find

no differences between growth forms – elevated CO_2 has the same stimulative effect on both lianas and trees.

We assessed the response of liana and tree seedlings and young saplings to elevated CO_2, therefore our conclusions are limited to this life stage. Recent research that found evidence of increasing lianas in tropical forests was conducted on adult stems (Schnitzer & Bongers, 2011). However, if elevated CO_2 were the main mechanism driving an increase in the size and abundance of lianas relative to trees we would expect at least some effect at earlier life stages. The reported increase in lianas likely is not limited to the adult life stage, because Benitez-Malvido & Martinez-Ramos (2003) found a 500% increase in liana seedling recruitment over a six and a half year period (1993-1999) in a Brazilian lowland moist forest.

While the interaction between elevated CO_2 and light availability was not included in our experimental design, we acknowledge its importance. Granados & Korner (2002), the only published work on tropical liana response to elevated CO_2 and light, found that lianas only increased in biomass under elevated CO_2 when grown under low light. In addition, three extratropical studies have found a larger liana response to CO_2 under low light (Korner, 2009). This advantage when light is limiting may allow lianas to escape the low-light understory and proliferate in the high light canopy faster than trees can. However, total daily average PAR in the wet-dry study and the low light level of Granados & Korner (2002) were similar (1.6 and 1.8 mol m^{-2}, respectively). Since neither study achieved the low-light level of the understory of a closed canopy neotropical forest (0.2-1.0 mol m^{-2}; Chazdon & Fetcher, 1983), further study of the interaction between understory light levels, plant growth form, and elevated CO_2 is needed.

Our results for the 11 liana and 10 tree species are reported at the growth form level, however species-specific response to CO_2 are not uniform. For example, in the dry-only

experiment, the liana *Stigmaphyllon lindenianum* increased in biomass 322% under elevated CO_2 relative to ambient, while the liana *Paullinia pinnata* showed a biomass decrease of 19%. In the same study, the tree *Cedrela odorata* increased in biomass 111% under elevated CO_2 relative to ambient, while the tree *Paquira quinata* showed a biomass decrease of 15%. The large species-level variation of lianas in response to CO_2 (Figures 2.2 and 2.3) led to a lack of any significant differences at the growth form level. Lianas are a diverse plant growth form in the neotropics with 162 species from 36 families present on the 50-ha plot alone at Barro Colorado Island in Panama (Schnitzer *et al*., 2012), so it is unreasonable to expect they would respond uniformly. It is possible that the reported increase in liana size and abundance is caused by a subset of species that differ among regions of the neotropics. Unfortunately, temporal censuses of lianas to date have not included species-level data. Not only are temporal species censuses needed, but any further study of lianas under elevated CO_2 should be focused on those liana species that do increase over time.

We conclude that elevated CO_2 is unlikely the main mechanism behind the reported liana increase, yet we cannot rule it out entirely. Other global change mechanisms such as increasing length and severity of seasonal drought, changes in soil nutrient cycles, and changes in temperature may interact with increasing atmospheric CO_2 to produce the reported increase in lianas. As with any perturbation to a natural system the underlying mechanisms and their effects on ecosystems are likely to be complex and interactive. Further experimentation on the mechanisms underlying increasing lianas in the neotropics should therefore be multifactorial and include species selected based on the results of temporal censuses.

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	Lianas			Trees	
Experiment	Family	Species	Family	Species	
Dry-only	Boraginaceae	*Tournefortia hirsutissima* L.	Apocynaceae	*Lacmelia panamensis*	
Dry-only	Celastraceae	*Prionostemma asperum* (Lam.) Miers	Malvaceae	*Paquira quinata (Jacq.)* W.S. Alverson	
Dry-only	Connaraceae	*Connarus turczaninowii* Triana	**Combretaceae**	***Terminalia amazonia*** (J.F. Gmel.) Exell	
Dry-only	Dilleniaceae	*Davilla kunthii* A. St.-Hil.	Fabaceae (Faboideae)	*Vatairea erythrocarpa* (Ducke) Ducke	
Dry-only	Loganiaceae	*Strychnos panamensis* Seem.	Meliaceae	*Cedrela odorata* L.	
Dry-only	Malpighiaceae	*Stigmaphyllon lindenianum* A. Juss.	Moraceae	*Brosimum alicastrum* Sw.	
Dry-only	Sapindaceae	*Paullinia pinnata* L.	**Rubiaceae**	***Calycophyllum candidissimum*** (Vahl) DC.	
Dry-only	Vitaceae	*Vitis tiliifolia* Humb. & Bonpl. ex Schult.	Rubiaceae	*Randia armata* (Sw.) DC.	
Wet-dry	Bignoniaceae	*Bignonia corymbosa* (Vent.) L.G. Lohmann	Bignoniaceae	*Tabebuia rosea* (Bertol.) A. DC.	
Wet-dry	Connaraceae	*Connarus sp.*	Boraginaceae	*Cordia alliodora* (Ruiz & Pav.) Oken	
Wet-dry	Fabaceae (Faboideae)	*Clitoria javitensis* (Kunth) Benth.	**Combretaceae**	***Terminalia amazonia*** (J.F. Gmel.) Exell	
Wet-dry	Malpighiaceae	*Stigmaphyllon hypargyreum* Triana & Planch.	**Rubiaceae**	***Calycophyllum candidissimum*** (Vahl) DC.	

Table 2.1. Species (listed by family) used in the two experiments. Species in bold indicate those used in both studies.

Response Variable	Experiment			
	Dry-only	Wet-dry	Wet-half	Dry-half
Growth Change				
Height (cm)	●	●	●	●
Diameter (cm)	●	●	●	●
Leaf Area (cm²)	●	●	●	●
Total Leaf Production	●	●	●	●
Leaf Loss	●	●	●	●
Biomass Change				
Leaf Biomass (g)	●	●		
Stem Biomass (g)	●	●		
Root Biomass (g)	●	●		
Total Biomass (g)	●	●	●	●
Relative Growth Rate		●	●	●
Ratios				
Leaf Area Ratio (cm² mg⁻¹)	●	●	●	●
Leaf Mass Area (mg cm⁻²)	●	●		
Specific Leaf Area (cm² mg⁻¹)	●	●		
Root:Shoot Ratio	●	●		
Leaf:Stem Ratio	●	●		
Physiology				
Max Photosynthetic Rate (μmol CO_2 m² s⁻¹)	●		●	●
Stomatal Conductance (mol H_2O m² s⁻¹)	●		●	●
Transpiration (mmol H_2O m² s⁻¹)	●		●	●
Foliar C:N ratio		●		

Table 2.2. Variables measured in the experiments and used as the response variables in the model, broken down by variable category and experiment. Note: the Wet-half and Dry-half experiments are subsets of the Wet-Dry experiment.

Response Variable	Dry-only				Wet-dry				Wet-half				Dry-half			
	Interaction	Main Effect		Random structure[1]	Interaction	Main Effect		Random structure[1]	Interaction	Main Effect		Random structure[1]	Interaction	Main Effect		Random structure[1]
		CO_2	GF			CO_2	GF			CO_2	GF			CO_2	GF	
Growth Change																
Stem Length (cm)	n.s.	n.s.	n.s.	2	n.s.	n.s.	n.s.	1	n.s.	n.s.	n.s.	1	n.s.	n.s.	n.s.	2
Diameter (cm)	n.s.	**0.040**	n.s.	2	n.s.	n.s.	n.s.	1	n.s.	n.s.	n.s.	2	n.s.	n.s.	n.s.	1
Leaf Area (cm^2)	n.s.	n.s.	n.s.	1	n.s.	**0.038**	n.s.	2	n.s.	n.s.	n.s.	2	n.s.	**0.034**	n.s.	2
Total Leaf Production	n.s.	n.s.	n.s.	1	n.s.	n.s.	n.s.	1	n.s.	n.s.	**0.035**	2	n.s.	n.s.	n.s.	1
Leaf Loss	n.s.	n.s.	n.s.	2	n.s.	n.s.	n.s.	2	n.s.	n.s.	n.s.	2	n.s.	n.s.	n.s.	2
Biomass Change																
Leaf Biomass (g)	n.s.	n.s.	n.s.	1	n.s.	**0.008**	n.s.	2	-	-	-	-	-	-	-	-
Stem Biomass (g)	n.s.	n.s.	n.s.	1	n.s.	**0.007**	n.s.	2	-	-	-	-	-	-	-	-
Root Biomass (g)	n.s.	**0.018**	n.s.	1	n.s.	n.s.	n.s.	2	-	-	-	-	-	-	-	-
Total Biomass (g)	n.s.	n.s.	n.s.	1	n.s.	**0.012**	n.s.	2	n.s.	n.s.	n.s.	2	n.s.	**0.017**	n.s.	2
Relative Growth Rate	-	-	-	-	n.s.	n.s.	n.s.	1	n.s.	n.s.	n.s.	1	n.s.	**0.044**	n.s.	1
Allocation Ratios																
Leaf Area Ratio (cm^2 mg^{-1})	n.s.	n.s.	n.s.	1	n.s.	n.s.	**0.016**	2	n.s.	n.s.	n.s.	1	n.s.	n.s.	n.s.	2
Leaf Mass Area (mg cm^{-2})	n.s.	**0.025**	n.s.	2	n.s.	n.s.	n.s.	2	-	-	-	-	-	-	-	-
Specific Leaf Area (cm^2 mg^{-1})	n.s.	n.s.	n.s.	2	n.s.	n.s.	n.s.	2	-	-	-	-	-	-	-	-
Root:Shoot Ratio	n.s.	n.s.	n.s.	2	n.s.	n.s.	n.s.	2	-	-	-	-	-	-	-	-
Leaf:Stem Ratio	n.s.	n.s.	n.s.	2	n.s.	n.s.	n.s.	2	-	-	-	-	-	-	-	-
Physiology																
Max Photosynthetic Rate (μmol CO_2 m^2 s^{-1})	n.s.	**<0.001**	n.s.	1	-	-	-	-	n.s.	**<0.001**	n.s.	1	n.s.	**<0.001**	n.s.	1
Stomatal Conductance (mol H_2O m^2 s^{-1})	n.s.	n.s.	n.s.	2	-	-	-	-	n.s.	n.s.	n.s.	1	n.s.	**0.001**	n.s.	1
Transpiration (mmol H_2O m^2 s^{-1})	n.s.	n.s.	**0.038**	1	-	-	-	-	n.s.	n.s.	n.s.	1	n.s.	**0.019**	n.s.	1
Foliar C:N ratio	-	-	-	-	n.s.	n.s.	n.s.	2	-	-	-	-	-	-	-	-

[1]Random effects structures:
1: ($\beta_{Chamber(i)}$ + $\gamma_{Species(i)}$)
2: ($\gamma_{Species(i)}$)

Table 2.3. Likelihood ratio test results for the interaction between CO_2 and growth form (GF) and for a main effect of CO_2 and GF separately. The random effects structure used for each model is given (see table footnotes for description). Significant effects are highlighted in bold, n.s. denotes non-significant effects, and - indicates variables not measured in a particular experiment or subset. Note: the Wet-half and Dry-half experiments are subsets of the Wet-Dry experiment.

Response Variable	Dry-only — Lianas				Dry-only — Trees				Wet-dry — Lianas				Wet-dry — Trees			
	Ambient	Elevated	% Δ	Effect Size	Ambient	Elevated	% Δ	Effect Size	Ambient	Elevated	% Δ	Effect Size	Ambient	Elevated	% Δ	Effect Size
Growth Change																
Stem Length (cm) — mean	20.0	27.0	35.2	0.30	6.0	8.4	41.0	0.34	89.8	108.1	20.4	0.19	21.3	25.53	19.7	0.18
Stem Length (cm) — SE	6.9	6.9		CI95 ± 0.84	6.9	6.9		CI95 ± 2.78	41.0	41.0		CI95 ± 1.16	41.0	41.0		CI95 ± 4.91
Diameter (cm)	0.54	0.55	3.1	0.03	0.51	0.48	-5.7	-0.06	0.15	0.18	20.1	0.18	0.18	0.21	17.0	0.16
	0.04	0.04		0.22	0.04	0.04		0.25	0.05	0.05		0.89	0.05	0.05		0.73
Leaf Area (cm²)	153.7	240.2	56.3	0.45	198.8	293.3	47.5	0.39	802.8	1086.7	35.4	0.30	651.3	823.9	26.5	0.23
	89.8	90.2		1.36	89.9	90.0		1.07	442.2	441.8		1.34	442.2	442.4		1.70
Total Leaf Production	4.9	10.3	108.4	0.73	4.5	6.0	31.8	0.28	18.5	27.4	47.5	0.39	31.2	35.5	13.8	0.13
	2.8	2.9		1.25	2.8	2.8		1.54	13.2	13.2		1.68	13.2	13.2		1.10
Leaf Loss	1.5	1.7	11.3	0.11	1.7	1.3	-20.5	-0.23	3.2	3.4	6.4	0.06	8.4	7.5	-10.1	-0.11
	0.55	0.55		0.95	0.55	0.55		1.03	1.9	1.9		1.55	1.9	1.9		0.65
Biomass Change																
Leaf Biomass (g)	0.51	0.91	78.8	0.58	0.60	0.85	42.6	0.35	2.26	3.66	61.4	0.48	1.80	2.40	32.9	0.28
	0.23	0.23		1.02	0.23	0.23		0.93	1.30	1.30		1.32	1..30	1.30		1.77
Stem Biomass (g)	0.36	0.72	98.8	0.69	0.43	0.69	60.0	0.47	2.65	5.18	95.9	0.67	1.09	1.68	32.9	0.28
	0.18	0.18		1.11	0.18	0.18		0.98	1.30	1.30		1.32	1.30	1.30		1.77
Root Biomass (g)	0.40	0.62	55.3	0.44	0.47	0.57	22.4	0.20	1.63	2.52	54.4	0.43	0.64	1.12	75.8	0.56
	0.14	0.14		0.82	0.14	0.14		0.77	1.14	1.14		1.63	1.14	1.14		4.04
Total Biomass (g)	1.27	2.24	76.4	0.57	1.49	2.12	41.8	0.35	6.64	11.36	73.8	0.55	3.52	5.18	46.9	0.38
	0.46	0.46		0.82	0.46	0.46		0.74	4.26	4.25		1.47	4.26	4.26		2.87
Relative Growth Rate	-	-	-	-	-	-	-	-	0.012	0.013	8.8	0.08	0.010	0.011	6.9	0.07
									0.003	0.003		0.71	0.003	0.003		0.74
Ratios																
Leaf Area Ratio (cm² g⁻¹)	0.12	0.11	-8.5	-0.09	0.11	0.10	-10.8	-0.11	0.13	0.12	-11.4	-0.12	0.18	0.17	-9.1	-0.10
	0.02	0.02		0.54	0.02	0.02		0.58	0.02	0.02		0.43	0.02	0.02		0.31
Leaf Mass Area (mg cm⁻²)	3.89	4.12	6.0	0.06	3.82	4.01	4.9	0.05	3.46	3.89	12.4	0.12	3.56	31.8	-10.8	-0.11
	0.42	0.42		0.29	0.42	0.42		0.30	0.51	0.51		0.39	0.51	0.51		0.42
Specific Leaf Area (cm² mg⁻¹)	0.30	0.29	-5.2	-0.05	0.29	0.28	-4.5	-0.05	0.31	0.28	-10.3	-0.11	0.42	0.37	-11.6	-0.12
	0.38	0.38		0.35	0.38	0.38		0.37	0.05	0.05		0.51	0.05	0.05		0.39
Root:Shoot Ratio	1.72	1.82	6.1	0.06	1.25	1.17	-6.2	-0.06	0.33	0.26	-22.1	-0.25	0.30	0.34	14.7	0.14
	0.60	0.60		0.93	0.60	0.60		1.37	0.06	0.06		0.58	0.06	0.06		0.54
Leaf:Stem Ratio	1.59	1.56	-1.8	-0.02	1.29	1.30	1.0	0.01	1.26	1.20	-5.4	-0.06	1.77	1.69	-4.3	-0.04
	0.32	0.32		0.56	0.32	0.32		0.68	0.30	0.30		0.67	0.30	0.30		0.48
Physiology																
Max Photosynthetic Rate (μmol CO₂ m² s⁻¹)	5.87	8.01	36.5	0.31	5.05	6.96	38.0	0.32	-	-	-	-	-	-	-	-
	0.62	0.62		0.26	0.61	0.62		0.29								
Stomatal Conductance (mol H₂O m² s⁻¹)	0.15	0.15	-0.7	-0.01	0.11	0.08	-26.2	-0.30	-	-	-	-	-	-	-	-
	0.02	0.02		0.39	0.02	0.02		0.64								
Transpiration (mmol H₂O m² s⁻¹)	2.21	2.14	-3.0	-0.03	1.77	1.40	-20.8	-0.23	-	-	-	-	-	-	-	-
	0.25	0.25		0.31	0.25	0.25		0.44								
Foliar C:N ratio	-	-	-	-	-	-	-	-	17.4	18.3	5.4	0.05	13.7	14.3	4.7	0.05
									2.9	2.9		0.45	2.9	2.9		0.57

Table 2.4. Mixed model estimates of liana and tree response to CO_2 treatment, percent change of response, and effect size for growth, biomass, response ratio, and physiological variables between the dry-only and wet-dry experiments. These values take into account the environmental covariates and random effects used in the model. - indicates variables not measured in a particular experiment.

|		Wet-half								Dry-half							
Response Variable		Lianas				Trees				Lianas				Trees			
		Ambient	Elevated	% Δ	Effect Size	Ambient	Elevated	% Δ	Effect Size	Ambient	Elevated	% Δ	Effect Size	Ambient	Elevated	% Δ	Effect Size
Growth Change																	
Stem Length (cm)	mean	35.8	31.2	21.0	0.19	5.9	6.4	8.4	0.08	63.4	76.5	20.6	0.19	15.6	20.2	29.9	0.26
	SE	15.0	15.0		±CI95 1.50	15.0	15.0		±CI95 6.88	27.5	27.5		±CI95 1.11	27.5	27.5		±CI95 4.40
Diameter (cm)		0.05	0.05	15.3	0.14	0.05	0.06	15.4	0.14	0.10	0.12	18.1	0.17	0.13	0.15	17.2	0.16
		0.02	0.02		0.92	0.02	0.02		0.83	0.04	0.04		0.91	0.04	0.04		0.68
Leaf Area (cm2)		192.4	217.3	12.9	0.12	247.0	253.1	2.5	0.02	630.9	862.8	36.7	0.31	415.8	572.5	37.7	0.32
		67.1	67.0		0.91	67.1	67.1		0.74	389.9	389.4		1.50	389.9	389.9		2.27
Total Leaf Production		3.9	4.3	9.8	0.09	9.9	10.5	5.9	0.06	14.9	23.5	57.7	0.46	21.3	25.9	21.8	0.20
		2.1	2.1		1.44	2.1	2.1		0.57	11.7	11.7		1.82	11.7	11.7		1.39
Leaf Loss		0.7	0.9	26.4	0.23	1.8	2.1	17.4	0.16	2.6	2.7	3.0	0.03	6.9	5.7	-17.3	-0.19
		0.5	0.5		1.90	0.5	0.5		0.74	1.5	1.5		1.61	1.5	1.5		0.68
Biomass Change																	
Leaf Biomass (g)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stem Biomass (g)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Root Biomass (g)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total Biomass (g)		1.13	1.32	16.4	0.15	0.98	1.00	2.9	0.03	5.72	9.78	71.0	0.54	2.23	3.70	65.4	0.50
		0.40	0.40		0.91	0.34	0.34		0.96	4.05	4.05		4.14	4.05	4.05		1.60
Relative Growth Rate		0.014	0.015	4.8	0.05	0.014	0.014	1.8	0.02	0.009	0.011	17.0	0.16	0.007	0.009	21.6	0.20
		0.004	0.004		0.77	0.004	0.004		0.69	0.003	0.003		0.71	0.003	0.003		0.87
Ratios																	
Leaf Area Ratio (cm2 g-1)		0.013	0.013	-0.9	0.01	0.019	0.019	1.4	0.01	0.082	0.075	-7.8	-0.08	0.104	0.103	-1.3	-0.01
		0.03	0.03		0.58	0.03	0.03		0.42	0.021	0.021		0.73	0.021	0.021		0.56
Leaf Mass Area (mg cm-2)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Specific Leaf Area (cm2 mg-1)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Root:Shoot Ratio		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leaf:Stem Ratio		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Physiology																	
Max Photosynthetic Rate (μmol CO_2 m2 s-1)		5.56	7.95	42.8	0.36	5.86	7.58	29.5	0.26	6.21	8.88	42.9	0.36	5.73	8.84	54.0	0.43
		0.68	0.68		0.29	0.68	0.68		0.29	1.30	1.30		0.50	1.30	1.31		0.53
Stomatal Conductance (mol H_2O m2 s-1)		0.12	0.14	17.7	0.16	0.15	0.17	10.2	0.10	0.10	0.06	-37.9	-0.48	0.11	0.09	-19.7	-0.22
		0.03	0.03		0.65	0.03	0.03		0.50	0.02	0.02		0.86	0.02	0.02		0.71
Transpiration (mmol H_2O m2 s-1)		1.50	1.71	14.0	0.13	1.89	2.00	6.4	0.06	1.69	1.26	-25.7	-0.30	1.79	1.61	-9.9	-0.10
		0.25	0.24		0.43	0.25	0.25		0.35	0.34	0.34		0.67	0.34	0.34		0.56
Foliar C:N ratio		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2.5. Mixed model estimates of liana and tree response to CO_2 treatment, percent change of response, and effect size for growth, biomass, response ratio, and physiological variables between the wet-half and dry-half of the wet-dry experiment. These values take into account the environmental covariates and random effects used in the model. - indicates variables not measured in a particular subset of the experiment.
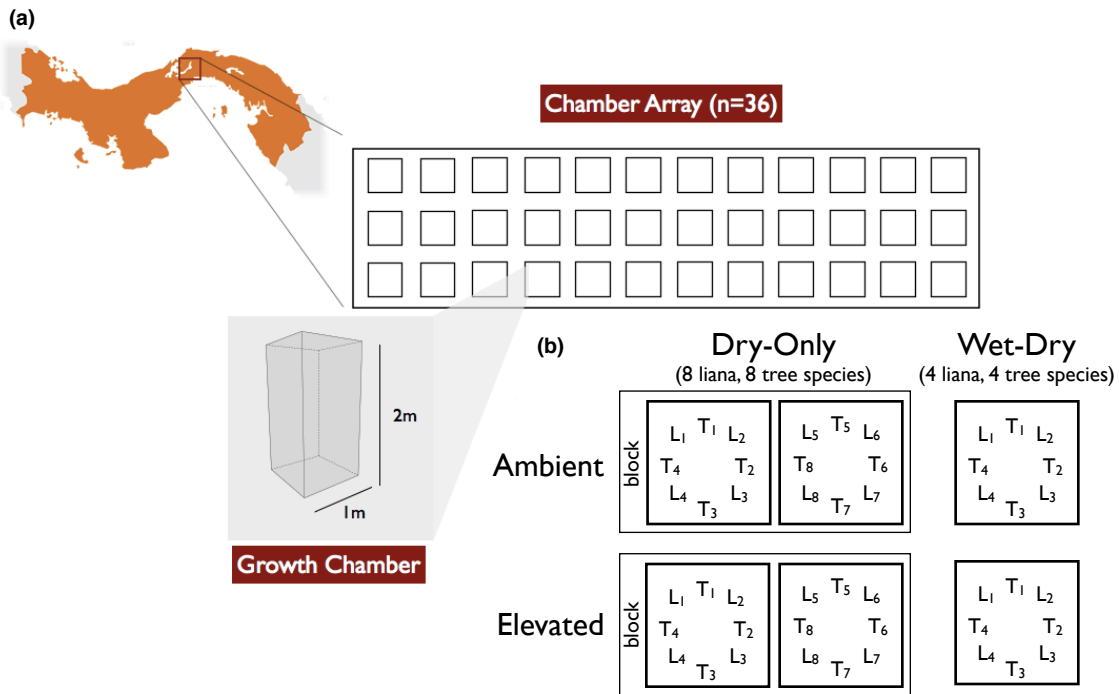
Figure 2.1. a) Open top chamber array location, layout, and dimensions. b) experimental design and species distribution among CO_2 treatments and chambers for each experiment. L = liana, T = tree; each subscript number represents a distinct species. Species locations within each chamber for both experiments, and between chambers within block for the dry-only experiment, were randomized before planting.
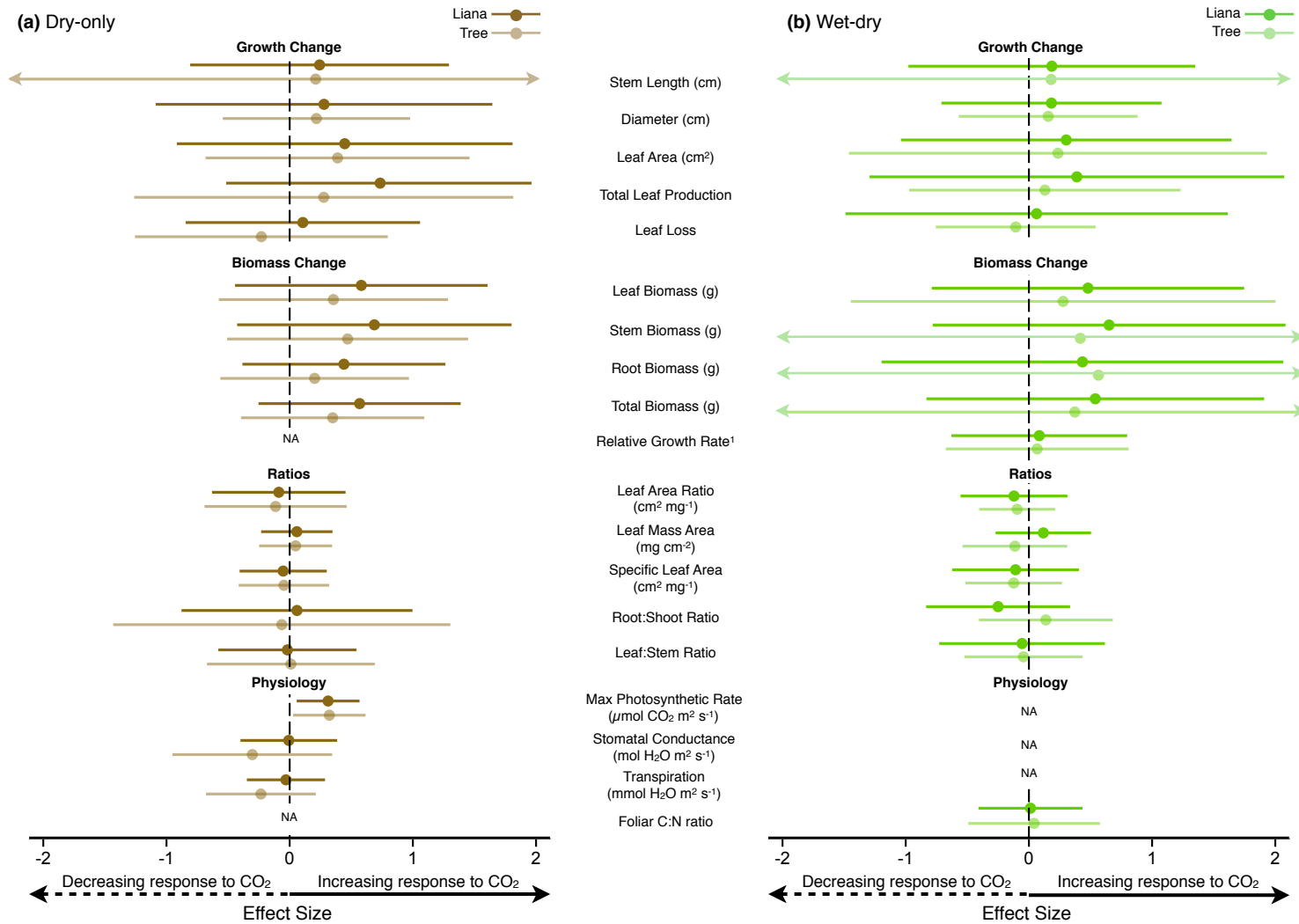
Figure 2.2. Effect size response to CO_2 for growth variables, biomass variables, response variable ratios, and physiological variables in the a) dry-only and b) wet-dry experiments. Positive/negative effect sizes indicate an increased/decreased response to CO_2. Points represent the mean effect size, lines represent the 95% confidence interval. Arrows denote confidence intervals that extend beyond the boundaries of the figure.

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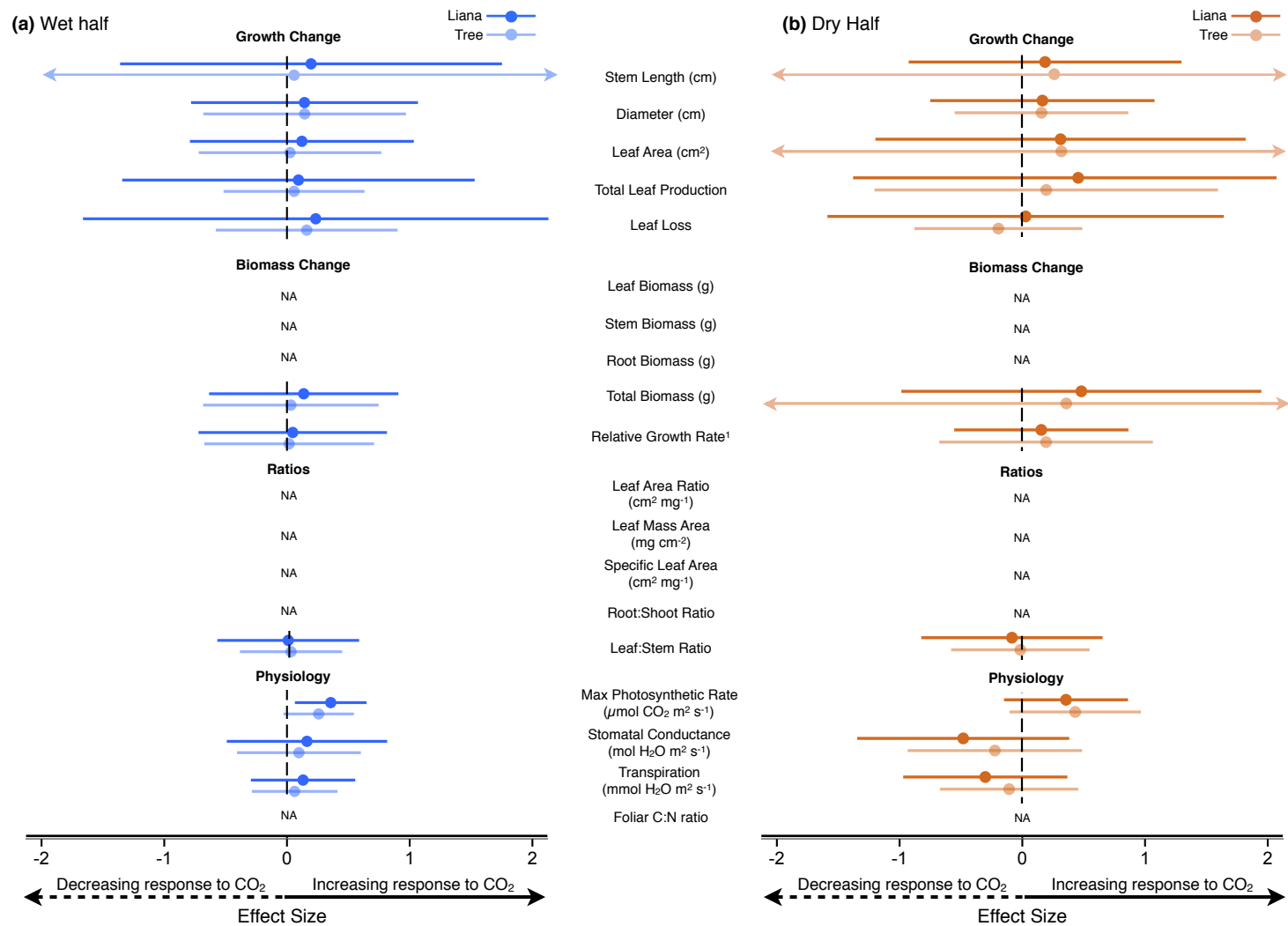
Figure 2.3. Effect size response to CO_2 for growth variables, biomass variables, response variable ratios, and physiological variables in the a) wet-half and b) dry-half of the wet-dry season experiment. Positive/negative effect sizes indicate an increased/decreased response to CO_2. Points represent the mean effect size, lines represent the 95% confidence interval. Arrows denote confidence intervals that extend beyond the boundaries of the figure.

Supplemental Methods

Chamber array and site details

Each chamber measured 1m x 1m x 2m (l x w x h) with frames constructed of ½ inch PVC pipe, wrapped with clear 8-gauge PVC film. Chambers were wrapped with 90% neutral density shade cloth to regulate incoming light levels and internal temperature. Chambers were arranged in three rows running west to east, each separated from the nearest chamber by 1.5 m. Chambers received a constant input of ambient air supplied by one of three industrial 51 cm panel fans (Multifan V4E5006, Vostermans Ventilation), attached to a plastic cylindrical plenum (4 m length x 1 m diameter). Each chamber was supplied from a plenum via flexible clothes dryer ducting (10 cm diameter) with the flow rate adjusted by metal duct dampening collars, resulting in a full air exchange approximately once every two minutes. Air entered the chambers from the ducting through 12 cm diameter PVC tee-junctions that split the airflow into each chamber at 90-degree angles and prevented direct airflow onto the plants. We randomly assigned half of the chambers to receive pure CO_2 at the air duct entrance. We regulated concentrations of CO_2 in each elevated treatment chamber to ≥ 700 μmol mol^{-1} through a bank of manual flow meters (Gilmont Industrial Flowmeter 200SML/min, Thermo Scientific) from sunrise to sunset (see below for details on CO_2 monitoring).

We constructed the experimental array next to a secondary forest edge in an area that may contain excavated or dredged soil from the construction of the Panama Canal 100 years ago (J. Wright, pers comm). The soil approximates native soils from a nearby undisturbed site. We determined total leaf mineral elements (Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn) by nitric acid digestion and ICP-OES (inductively coupled plasma atomic emission spectroscopy) detection in

a subset of individuals (two per species, per treatment). Elemental concentrations in the leaves of the plants showed no abnormal accumulation or deficiency, compared with data from 300 species on the 50 ha plot of Barro Colorado Island (B. Turner, pers comm).

Environmental monitoring

CO₂: An automatic sampling system monitored concentrations of CO_2 in all 18 elevated chambers and two ambient chambers. One of two diaphragm pumps (2107 Vacuum Pump, Thomas) pulled air from a single chamber and passed it into a 5 L buffer volume containing an infrared gas analyzer (GMW20, Vaisala) for a five-minute period. Two banks of solenoids powered by a microcontroller (Mega, Arduino) switched the sample stream among the chambers such that each chamber was sampled 5-6 times during the day. CO_2 concentrations were recorded to a datalogger (CR10X, Campbell Scientific) at the end of each five-minute period (Table S2.1).

Soil moisture: Three volumetric water content (VWC) meters (EC-5, Decagon Devices) continuously monitored moisture from 2-7 cm soil depth and recorded hourly averages to the datalogger throughout the wet-dry season experiment. Two of the sensors were permanently installed inside two chambers, while one rotated every three days among all the other chambers. During the dry-only experiment a VWC probe (CS616, Campbell Scientific) was used to spot check moisture in the upper 20 cm of the soil in each chamber weekly (Table S2.1).

Light: In the wet-dry season experiment two quantum sensors (LI190SB, Campbell Scientific) continuously monitored photosynthetically active radiation (PAR) and recorded five-minute averages to the datalogger. One sensor was permanently installed in a chamber in the middle of the array, while the other rotated every three days among all the chambers. In the dry-only experiment one quantum sensor was used to spot check light levels inside every chamber

during periods of full sun twice throughout the experiment. Light levels in each chamber as a percentage of full sun were calculated. To calculate daily total PAR (mol m^{-2}), full-sun average daily PAR was multiplied by individual chamber percentage light level (Table S2.1).

Temperature: Twenty-four thermocouples (Type T, Omega Engineering) were evenly spaced throughout the array (half inside and half outside the chambers) and recorded five-minute averages through a multiplexer (AM416, Campbell Scientific) linked to the datalogger (Table S2.1).

Soil nutrients: Soil samples were processed and analyzed at the STRI Soils Laboratory in Panama City, Panama. Ammonium and nitrate concentrations were determined by KCl extraction and automated colorimetry. Phosphorus concentrations were determined by anion exchange membrane extraction and automated molybdate colorimetry.

Plant biomass allometry

We calculated allometric estimates of plant total biomass at the beginning of both experiments and at the end of the wet season for the wet-dry experiment for each species using the equation:

$$\ln(y) = \beta_1 X_1^{\beta_2} + X_1^{\beta_3} + \cdots + \beta_{n1} X_n^{\beta_{n2}} + X_n^{\beta_{n3}} + \varepsilon \tag{1}$$

where y is the response variable (biomass), X_n is a predictor variable, β_{n1} and β_{n2} are regression coefficients, β_{n3} is a coefficient that allows the errors to scale with the predictor variables (Mascaro *et al.*, 2011), and e is the regression error term. We fit this model using a non-linear maximum likelihood optimization function (R function 'optim'). We compare full models with all predictor variables (leaf area, leaf number, diameter, and height) to nested models, and the model with the lowest corrected AIC (i.e., for sample size) value was chosen.

We used coefficients from this best-fit model to predict the initial and end-of-wet season biomass of the plants grown in the experiments.

We developed simple linear regression equations for each species to predict the mid-point leaf area from the length and width measurements taken from each leaf or leaflet 1) in the field halfway through the study and 2) in the lab at harvest.

Site abiotic variable modeling

We modeled light levels (PAR 5-min averages) in each chamber throughout the wet-dry experiment using a non-linear maximum likelihood optimization function (R function 'optim') to fit the overlapping data from the roving and stationary light sensors. We used an asymptotic model of the form:

$$y = \beta_1 * \left(1 - e^{-\beta_2 X^{\beta_3}}\right) \tag{2}$$

where y is the light level from the roving sensor, X is the light level from the stationary sensor, and β_1, β_2, and β_3 are the model coefficients. We used the model coefficients to predict light values during times the roving sensor was not present in each chamber. We used the same process to predict soil moisture from the permanent and roving VWC sensors.

Experimental Period		Elevated CO$_2$ (ppm)	Ambient CO$_2$ (ppm)	VWC	Monthly Precipitation (mm)	Light (Total Daily PAR)	Temperature (°C)
Dry-only	Mean	759	387	0.29	68	5.17	31.2
	SD	214	38	0.02		4.53	5.3
Wet		733	388	0.39	381	1.18	26.4
		203	40	0.03		0.45	4.6
Dry		699	367	0.30	7*	1.95	29.2
		214	28	0.06		0.39	5.5

*Missing data 1-28-2012 to 2-20-2012

Table S2.1 – Mean and standard deviation for each experimental period of all environmental variables recorded inside the chambers (except precipitation, which was recorded at the nearby monitoring station). VWC = soil volumetric water content. PAR = photosynthetically active radiation (mol m^{-2}).

Chapter III

The relative growth response of tropical lianas to elevated CO$_2$ does not depend on soil nutrient availability[*]

Abstract

A potential cause of increasing liana size and abundance relative to trees in neotropical

forests is the change in global biogeochemical cycles. Increasing atmospheric CO$_2$, higher rates

of nitrogen deposition, and decreasing soil phosphorus levels may interact to give tropical lianas

an advantage over trees. If lianas continue to increase, they may continue to change these

biogeochemical cycles by reducing tropical forest carbon storage and redistributing nutrients at

local scales. We investigated the growth response of locally abundant tropical liana and tree

species grown in open-top chambers in Panama, half of which were maintained at twice-ambient

levels of CO$_2$. In two separate studies, seedlings of two liana and two tree species were grown

with reduced soil nitrogen (N), and seedlings of three liana species were grown with reduced soil

phosphorus (P). Half the pots in each experiment received weekly additions of a nutrient

mixture. Our experiments showed no evidence that either soil N or soil P availability interact

with increasing atmospheric CO$_2$ to significantly affect the growth and physiology of the species

studied. Instead, increases in soil nutrient availability or in CO$_2$ alone had both strong and

significant effects on the growth response of the species studied. For both the N and P

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experiments, higher soil nutrient availability led to a larger number of significant differences in plant growth response between treatments than did increased CO_2. This suggests that while explanations for the reported liana increase may not lie in the interaction among changing biogeochemical cycles, changes within these cycles alone could be a potential contributor.

Introduction

The recently reported increase in the size and abundance of neotropical lianas (woody vines) relative to trees (Schnitzer & Bongers, 2011; Schnitzer *et al.*, 2012; Yorke *et al.*, 2013) has the potential to alter biogeochemical cycles of neotropical forests. Lianas have a disproportionate impact on the growth and mortality of trees relative to the overall liana contribution to forest biomass. While lianas comprise about 10% of aboveground forest biomass (Putz, 1983; DeWalt & Chave, 2004), lianas can reduce tree growth (Lowe & Walker, 1977; Whigham, 1984; Clark & Clark, 1990; Grauel & Putz, 2004; van der Heijden & Phillips, 2009) and they are associated with a 40-100% increase in tree mortality (Phillips *et al.*, 2002; Ingwell *et al.*, 2010). Consequently, lianas do not fully replace the forest biomass lost as a result of their negative effects on trees (Chave *et al.*, 2001; van der Heijden & Phillips, 2009), potentially altering the balance of carbon exchanged between the atmosphere and biosphere.

Nutrient cycles have the potential to change as a result of increasing liana size and abundance. Leaf nitrogen (N), phosphorus (P), and other nutrient (mass-based) concentrations are higher in lianas than trees (Asner & Martin, 2012), which could result in higher soil nutrients where lianas become more abundant. Liana canopies can grow tens of meters horizontally away from their root zone (Penalosa, 1984; Putz, 1984), thereby redistributing nutrients at a local scale (Powers & Kalicin, 2004).

Global scale alterations of biogeochemical cycles have been implicated as a potential cause of the relative increase in lianas (Zhu & Cao 2010; Schnitzer & Bongers, 2011). However, whether the increase in liana size and abundance relative to trees is a product of the interactions between increasing atmospheric CO_2 and changing nutrient cycles has yet to be empirically tested. Global atmospheric CO_2 levels have risen 40% since 1750 (IPCC, 2013), with most of the increase occurring since 1960 (NOAA, 2013). The relatively greater investment in photosynthetic tissue by lianas may allow them to take advantage of increased CO_2 levels to a greater extent than trees (Schnitzer & Bongers, 2011).

In addition to increasing CO_2 levels, moist tropical forests have experienced increased anthropogenic N deposition in the last 40 years (Hietz *et al.*, 2011) and are projected to receive the highest loadings of N deposition globally in coming decades (Galloway *et al.*, 2004). In lowland tropical forests, N is presumed to be in excess of plant demand (Hedin *et al.*, 2009; Brookshire *et al.*, 2012), however *in situ* fertilization experiments show that current soil N availability does in fact limit tree growth (Wright *et al.*, 2011). Lianas were not analyzed in these experiments, but there is evidence that liana density is positively correlated with soil fertility in the neotropics (Gentry, 1991). High liana abundance is often found in disturbed areas, which tend to have higher N availability resulting from increased N mineralization (Kazda & Salzer, 2000). Lianas have both higher foliar (Asner & Martin, 2012) and whole plant (Cernusak *et al.*, 2008) N concentrations than trees, so the association between high soil N availability and liana abundance suggests lianas may benefit from increased N deposition. The response of lianas to both elevated atmospheric CO_2 and higher available soil N may provide a mechanistic understanding of the reported increase of liana size and abundance.

Phosphorus availability, often assumed to be of key importance in constraining the productivity of lowland tropical forests, may decline as a consequence of increased N deposition (Matson *et al.*, 1999). Higher concentrations of N increase soil acidity, resulting in increased base cation losses and increased fixation of P into insoluble forms (Matson *et al.*, 1999). Liana abundance has been associated with high soil P availability (Laurance *et al.*, 2001; Malizia *et al.*, 2010), suggesting that a decline in soil P availability may constrain future increases of lianas. Under elevated atmospheric CO_2, soil P available to lianas may be further restricted by lower transpiration rates that result from higher plant water use efficiency (Battipaglia *et al.*, 2012; but see *Chapter 2*). Lower transpiration rates decrease mass-flow of soil water and dissolved P into roots, resulting in reduced acquisition by plants (Cramer & Hoffmann, 2008; Cernusak & Winter, 2011).

An alternative hypothesis put forth by Zhu & Cao (2010) argues that an interaction between elevated CO_2 and low soil P availability may actually give tropical lianas an advantage over trees. They hypothesize that the generally low soil P availability in tropical forests will constrain the CO_2 fertilization effect for all plants, but that lianas may be less constrained due to higher phosphorus-use efficiency than trees.

We tested the strength of interactions among carbon, nitrogen, and phosphorus in tropical forests to determine whether the interactions contribute to the reported increase in liana abundance. In two separate experiments, we investigated how soil nutrient (N and P) availability affected the response of lianas to increasing atmospheric CO_2. Tropical lianas and trees were grown in pots within open top plant growth chambers where they were exposed to either ambient or elevated levels of CO_2. One experiment manipulated soil N availability on both tree and liana species and the other manipulated soil P availability on liana species only. Each experiment held

other nutrients constant across treatments. We asked whether 1) higher levels of soil N availability lead to a larger growth response in lianas than in trees under elevated CO_2, and 2) lower soil P availability reduces the growth response of lianas under elevated CO_2 compared to ambient CO_2. We hypothesized that tropical lianas growing under elevated CO_2 would show 1) a larger relative growth response than trees at high soil N levels, and 2) a reduction in relative growth response when available P is low compared with growth under ambient CO_2.

Methods

Site and Species

We constructed an array of 36 open-top growth chambers along a forest edge at the Smithsonian Tropical Research Institute's (STRI) Experimental Plant Growth Facility in the Republic of Panama (Figure 3.1a). Chamber design and CO_2 delivery system are described in Chapter 2 *Materials and Methods*. Sensors monitored temperature and light inside a subset of chambers throughout each experiment. Additionally, STRI collects hourly readings of temperature, precipitation, and full-sun photosynthetically active radiation (PAR) at this site. Over the last seven years monthly average daytime temperature was 28.4 °C, average yearly precipitation was 2133 mm, and average daily total PAR was 28.0 mol m^{-2}.

We germinated seeds in trays (5 cm diameter x 9 cm height cells) in a shade house (covered with 70% shade cloth) and transplanted seedlings with at least one true leaf to 2.5 L pots (10 cm diameter x 30 cm height) 30 days before being placed in the chambers. Each pot included one individual, and we placed four pots in each chamber (Figure 3.1b). We conducted both experiments during a concurrent experiment with plants growing in the soil of each chamber plot (Chapter 2). Runoff from the pots was physically isolated to avoid nutrient transfer

to the chamber plot soil. We connected plastic containers (28 cm diameter x 30 cm height) isolating the four individual pots per chamber to a PVC pipe network that drained the containers downslope and away from the chamber array.

We used two liana and two tree species in the nitrogen experiment, and three liana species in the phosphorus experiment (Table 3.1). We selected species from among the most common species in central Panama (DeWalt *et al.*, 2000; Hubbell *et al.*, 2005; Schnitzer *et al.*, 2012), and based on the availability of fruits, seeds, and seedlings from Barro Colorado Nature Monument forests and local reforestation nurseries.

Experimental Design

We used a balanced factorial design for both nutrient manipulation experiments (Figure 3.1b), with nine individuals per species per treatment combination. Each pot in a chamber contained a distinct species in each experiment (Table 3.1). All pots in each chamber received the same nutrient treatment (either low or high) and CO_2 treatment (either elevated or ambient). Nutrient treatments were applied weekly to half the pots in each experiment and are described immediately below. Both experiments ran for 60 days.

Nitrogen Experiment

The nitrogen experiment ("N experiment") ran from September to November 2011. We used a volumetric mixture of 80% soil and 20% rice husks as the growing medium. By increasing the carbon to nitrogen (C:N) ratio of the soil, rice husks reduce available soil N to plants due to increased microbial immobilization of nitrate (Dalling *et al.*, 2013). Soil was collected from the top 10 cm of a nearby orchard, air-dried, and passed through a 1 cm sieve to remove large rocks and organic debris. The soil is considered relatively fertile (see Dalling *et al.*, 2013).

To return the level of N in the high-N treatment pots to the original level of the soil (Dalling *et al.*, 2013), we added 150 ml of 11.2 mg N (from 24 mg urea) solution to each of these pots weekly ("high N treatment"). We added 150 ml of deionized water to the depleted N treatment pots weekly ("low N treatment"). Additional watering was not needed because this experiment was conducted during the wet season. Final concentrations of soil ammonium, nitrate, and P are shown in Table 3.2.

Phosphorus Experiment

The phosphorus experiment ("P experiment") ran from January to March 2012. The growing medium was a volumetric mixture of 60% acid washed river sand, 30% fine silica sand, and 10% soil (same soil as described in "Nitrogen Experiment" above). We used this sand-soil combination to reduce total nutrient availability while inoculating the growing medium with soil biota. All pots received a weekly addition of 200 ml nutrient solution that consisted of 72.0 mg N, 201.1 mg K (from 520 mg potassium nitrate), 61.1 mg Mg (from 232 mg magnesium sulfate), and 208.2 mg Ca (from 571 mg calcium chloride). The nutrient solution for the high P treatment contained an additional 21.2 mg of P (from 82 mg monosodium phosphate) to approximate soil P levels for the area (Garrish *et al.*, 2010). All pots also received 0.1 ml of a micronutrient solution and 0.1 ml of iron chelate solution. We watered all pots with 100 ml of deionized water 4 days after the nutrient solution addition to maintain soil moisture near typical wet season levels (c. 40% volumetric water content). Final concentrations of soil ammonium, nitrate, and P are shown in Table 3.2.

Plant and Soil Measurements

At the beginning of each experiment, we harvested 10 to 20 extra seedlings per species that were not used in the experiment and measured the height of the apical bud above soil (cm),

diameter at 5 cm height (mm), number of live leaves, and dry above- and belowground biomass (g). We used these data to estimate the biomass of the experimental seedlings allometrically at the start of the experiment using the equation:

$$\ln(y) = \beta_1 X_1^{\beta_2} + X_1^{\beta_3} + \cdots + \beta_{n1} X_n^{\beta_{n2}} + X_n^{\beta_{n3}} + \varepsilon \tag{1}$$

where y is the response variable (biomass), X_n is a predictor variable, β_{n1} and β_{n2} are regression coefficients, β_{n3} is a coefficient that allows the errors to scale with the predictor variables (Mascaro *et al.*, 2011), and ε is the regression error term. We fit this model using a non-linear maximum likelihood optimization function (R 'optim'; R Development Core Team, 2012). We compared the full models with all predictor variables (leaf number, diameter, and height) to nested models, and chose the model with the lowest corrected AIC value (i.e., corrected for sample size) value. We used the coefficients from this best-fit model to predict the initial biomass of the plants grown in the experiments.

We used initial biomass estimates to calculate the relative growth rate (RGR) of the biomass of each plant during the experiment:

$$RGR = \frac{\ln(M_{final}) - \ln(M_{init})}{t} \tag{2}$$

where M_{init} is the allometrically estimated dry biomass of each plant at the start of the treatment, M_{final} is the measured dry biomass at harvest, and t is the number of days between the treatment start and plant harvest.

Every fifteen days during both experiments we measured the stem diameter (at 5 cm above the soil) and height, and counted the number of live and dead leaves for each plant. One day before the end of each experiment, we collected gas exchange measurements from the newest fully-expanded leaf on each plant. We used a portable photosynthesis system (LI-COR 6400XT) to measured maximum photosynthetic rate (μmol CO_2 m^2 s^{-1}), stomatal conductance

(mol H_2O m^2 s^{-1}), and transpiration rate (mmol H_2O m^2 s^{-1}). Inside the leaf chamber of the

photosynthesis system, light levels were set to 1000 µmol m^{-2} s^{-1} PAR and CO_2 concentration set

to the appropriate chamber target level (i.e., 390 µmol mol^{-1} or 780 µmol mol^{-1}). Maximum (i.e.,

light saturated) photosynthetic rate is a commonly used parameter in studies evaluating changes

in photosynthetic capacity in response to elevated CO_2 (see Curtis & Wang 1998). In the P

experiment, we also measured nighttime respiration (µmol CO_2 m^2 s^{-1}), stomatal conductance,

and transpiration rates between 10 pm and 4 am using the same procedures as above but with the

leaf chamber light off.

At the end of each experiment, in addition to the final biweekly measurements, we

harvested all plants above and below ground, and separately weighed the dry biomass of leaves,

stems, and roots. Total leaf production is defined here as the difference between the number of

live leaves at the beginning and number at the end of the treatment, plus all dead leaves. Leaf

loss is calculated as the total number of dead leaves regardless of the mechanism (e.g.,

abscission, herbivory, pathogens).

We determined foliar carbon and nitrogen by combustion and thermal conductivity

detection using a Thermo Flash 1112 elemental analyzer (CE Elantech, New Jersey, USA).

Foliar P was determined by ignition (550C, 1 hour) and dissolution in 1 M H_2SO_4, with

phosphate detection by automated molybdate colorimetry using a Lacaht Quikchem 8500 flow

injection analyzer (Hach Ltd, Loveland, CO, USA). In both experiments we conducted root

phosphatase enzyme assays on fine roots from a subset of individuals per species from each

treatment (Table 2). Root phosphatase activity was determined in sodium acetate buffer (pH 5.0)

using para-nitrophenyl phosphate (pNP) as substrate (5 mM final concentration) and

spectrophotometric detection using a method similar to that of Turner *et al*. (2001). Stable

isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of leaves from the P experiment were determined simultaneously by isotope ratio mass spectrometry using a Flash HT Elemental Analyzer coupled through a Conflo III interface to a Delta V Advantage continuous flow isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany).

Homogenized soil samples from the top 10 cm of each pot were analyzed for extractable ammonium and nitrate by KCl extraction and automated colorimetry, and plant available P concentrations by anion exchange membrane extraction and automated molybdate colorimetry, as described previously (Turner and Romero, 2009).

Data Processing and Analysis

All data processing and analysis was performed in the open-source statistical software program R (R Development Core Team, 2012). To test each response variable for categorical treatment main effects and interactions we fit linear mixed-effects models with restricted maximum likelihood (REML) estimation (Pinhero & Bates, 2000) in the R package 'lme4' (Bates *et al.*, 2013). The plant response variables analyzed in each experiment are presented in Table 3.2. To partially account for chamber-to-chamber variability we used environmental variables measured within the growth chambers as covariates in the model. These included total chamber PAR and soil ammonium, nitrate, and phosphorus concentrations in each pot. To compare coefficients directly we standardized all covariates by subtracting the mean and dividing by two standard deviations (Gelman & Hill, 2007). Random effects were included for chamber to account for any extra-treatment variation not captured by the covariates, and for species to account for species variation not due to growth form and treatment.

For the N experiment, fixed effects were CO_2 treatment (levels: elevated and ambient), growth form (levels: liana and tree), nutrient treatment (levels: high and low N), and their interactions for i individuals in a model of the form:

$$\text{Response}_i = \alpha_{CO_2(i),GF(i),N(i)} + \delta_{\text{Covariates}(i)} + \left(\beta_{\text{Chamber}(i)} + \gamma_{\text{Species}(i)} \right) + \varepsilon_i$$

where Response_i is one of the measured plant response variables (Table 3.2), $\alpha_{CO_2(i),GF(i),N(i)}$ represents the set of regression coefficients for each treatment and their interactions, $\delta_{\text{Covariates}(i)}$ represents the environmental variables used as covariates, $\beta_{\text{Chamber}(i)}$ and $\gamma_{\text{Species}(i)}$ are crossed-random effects that allow the regression intercepts to vary, and ε_i are the residual errors.

For the P experiment, CO_2 treatment (elevated and ambient), nutrient treatment (high and low P), and their interaction were the fixed effects for i individuals in a model of the form:

$$\text{Response}_i = \alpha_{CO_2(i),P(i)} + \delta_{\text{Covariates}(i)} + \left(\beta_{\text{Chamber}(i)} + \gamma_{\text{Species}(i)} \right) + \varepsilon_i \qquad (4)$$

where each term is the same as (3) except growth form and its interaction with the other treatments was not included because only liana species were used in this experiment.

We tested one alternate random effects structure for the models with only $\gamma_{\text{Species}(i)}$ as the random intercept. We chose the optimal random effects structure for each response variable using likelihood ratio tests in a simplified model containing only covariates. When chamber-to-chamber variation was small to nonexistent, this alternate "species-only" random effects structure was selected.

To generate p-values for each model coefficient, we used code adapted from Moore (2010) that iteratively fits reduced fixed effects models and compares them to the full fixed effects model using a likelihood ratio test. These models are all fit using maximum likelihood estimation instead of REML because REML estimates are not comparable among models with different fixed effects structures (Pinhero & Bates, 2000). When the interaction or a main effect

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term was not significant, the term(s) were removed and the model refit using the same procedure above.

We used the R package 'lsmeans' (Lenth, 2013) to calculate the least squares means for each level of CO_2 and growth form in the interaction model. From these data we calculated the mean effect size (i.e., log response ratio) and the 95% confidence interval of the effect size separately for the liana and tree response to elevated CO_2, following the method of Hedges *et al.* (1999).

Results

Nitrogen Experiment

We found no significant differences between lianas and trees in their response to changes in CO_2 and soil N availability for all response variables measured. Results from the N experiment are presented as percent change in the response variable from ambient to elevated CO_2 in Table 3.4. Lianas tended to have similar positive responses to elevated CO_2 regardless of soil N availability for all growth and biomass response variables, however trees did not. While this three-way interaction was non-significant, trees consistently had a negative or reduced response to elevated CO_2 in the high compared to the low N treatment. This was seen across almost all of the growth and biomass response variables with the exception of RGR, and lends partial support to our hypothesis that lianas would show a larger relative growth response to elevated CO_2 with higher N availability than trees.

When compared to the growth response variables, a smaller number of physiological response variables had large differences between ambient and elevated CO_2 under low and high N treatments. Lianas and trees had very similar relative increases in maximum photosynthetic

rate in response to elevated CO_2 regardless of N availability. In contrast, stomatal conductance and transpiration increased in lianas and decreased for trees under elevated CO_2, but did not differ between N treatments. The production of root phosphatase, an N-rich enzyme excreted by plants to convert organic to usable inorganic P near the root surface (Houlton *et al.*, 2008) increased under elevated CO_2 for both growth forms. While non-significant, lianas tended to show a larger phosphatase increase in response to CO_2 than trees, especially under the low N treatment.

When growth form was removed from the model, and only the interaction between elevated CO_2 and N treatment was analyzed, we found no significant interactions for any variables (not shown). To demonstrate the presence of experimental treatment effects we review briefly the main effects of CO_2, N, and growth form below.

When pooled across N treatments and growth forms, elevated CO_2 led to a significant increase in plant diameter (63.1%) and root biomass (46.2%), compared to ambient CO_2 (Figure 3.2). There was also a significant main effect of CO_2 for maximum photosynthetic rate (31.5% increase) and transpiration (24.8% decrease), which suggests the plants increased their water use efficiency under elevated CO_2 (Figure 3.2).

We found a number of significant main effects of N treatment when CO_2 treatment and growth forms were pooled. Higher soil N availability led to a 34.3% increase in total leaf production and a 33.2% increase in the relative growth rate of plants (Figure 3.2). Significant increases were found for both leaf area ratio (14.6%) and leaf-to-stem mass ratio (29.1%), but the root-to-shoot ratio significantly decreased by 24.9% (Figure 3.2). Thus, higher N availability appears to shift biomass allocation aboveground, especially toward leaves for both lianas and trees independent of CO_2 levels. Plants in the high N treatment also significantly increased their

maximum photosynthetic rates by 27.0% and their root phosphatase activity by 27.1% (Figure 3.2).

Lianas and trees showed differences mainly in their allocation strategies when we pooled the data across CO_2 and N treatments. Trees had significantly higher leaf mass per area (79.0%) than lianas, which indicates higher investment by the trees in light capture. This was also reflected in a significantly higher (47.9%) leaf-to-stem mass ratio in trees than in lianas. Trees had substantially and significantly higher (184.1%) root phosphatase activity than did lianas.

For the majority of the response variables measured (15 of 20), the "species-only" random effects structure was selected by the likelihood ratio test, and not the crossed random effects structure including both chamber and species. This suggests that the environmental covariates measured throughout the experiment sufficiently explained any chamber-to-chamber variability, or there was little to no inherent chamber-to-chamber variability for most of the response variables.

Phosphorus Experiment

We present results from the P experiment as the percent change in the response variable from ambient to elevated CO_2 (Table 3.5). Similar to the N experiment, almost no interactions between the CO_2 and P treatments were found for the 26 response variables measured for the three liana species. The one significant exception was leaf loss: in the high P treatment significantly more leaves were retained (92.2%) in response to elevated CO_2, but in the low P treatment significantly more leaves were lost (120.7%) in response to elevated CO_2. Other notable but non-significant patterns between the treatments were the larger relative responses to elevated CO_2 in the low P compared to the high P treatment for stem height, diameter, total leaf production, and relative growth rate.

To demonstrate the presence of experimental treatment effects we review briefly the main effects of CO_2 and P treatments separately. Most of the significant differences between ambient and elevated CO_2 occurred at the leaf level when pooling across P treatment. Elevated CO_2 stimulated significant increases in leaf area (39.4%), leaf biomass (40.2%), leaf area ratio (15.9%), and maximum photosynthetic rate (49.1%) (Figure 3.3). The significant increase in $\delta^{13}C$ (3.1%) in response to elevated CO_2 indicates higher intrinsic water use efficiency at elevated compared to ambient CO_2 (Figure 3.3). Finally, there was a significant decrease (30.1%) in the root-to-shoot ratio in response to elevated CO_2 (Figure 3.3), indicating a shift in biomass allocation from below to above ground.

Many response variables showed a significant difference between P treatments when pooling across CO_2 treatments. A significant increase was found in stem height (33.0%), leaf area (43.5%), total leaf production (32.5%), leaf biomass (47.2%), stem biomass (42.3%), total biomass (42.3%), and relative growth rate (53.4%) between low and high P treatments (Figure 3.3). Leaf area ratio and leaf-to-stem ratio significantly increased by 21.5% and 24.9%, respectively, in response to higher P availability (Figure 3.3). Significant changes in leaf mass per area (16.4% decrease) and its inverse, specific leaf area (14.6% increase) (Figure 3.3), suggest that increased P allows lianas to invest more efficiently in light capture (Poorter & Bongers, 2006). Higher P availability also resulted in a number of significant physiological responses. Photosynthetic rate was significantly higher (23.7%), as was foliar P concentrations (24.5%) under the high P treatment (Figure 3.3). Both foliar C:N ratio and foliar $\delta^{15}N$ decreased significantly under the high P treatment (11.6% and 21.0% respectively) (Figure 3.3).

For most of the response variables, the "species-only" random effects structure was chosen by the likelihood ratio test. In only 5 of the 26 response variables did chamber-to-

chamber variability have an effect. This suggests that there was little inherent chamber-to-chamber variability or that the environmental covariates sufficiently captured most of the between chamber variability.

Discussion

Our results do not support our hypothesis that interactions between changing carbon, nitrogen, and phosphorus cycles are a prime contributor to the liana increase in neotropical forests. In our experiments, lianas and trees responded similarly in growth and physiological responses to elevated CO_2 at both N treatment levels, and lianas did not change their relative response to CO_2 between the P treatment levels. Based on the lack of any interactions between elevated CO_2 and soil nutrient availability in either the N or the P experiment (with the exception of leaf loss for P), we conclude that increasing CO_2, combined with either N deposition or P depletion, is unlikely the main underlying mechanism explaining the liana increase in size and abundance.

Lianas in both high and low N treatments and trees in the low N treatment responded positively to elevated CO_2. Conversely, we observed a strong pattern of negative or decreased tree growth and biomass response to elevated CO_2 under high N treatment. However, none of these response variables had a significant three-way interaction among growth form, CO_2, and N. Our hypothesis that we would find a larger relative growth response under elevated CO_2 and high N in lianas than in trees was not supported. The lack of an interaction between elevated CO_2 and higher N availability regardless of growth form stands in contrast to previous studies of woody plant response to CO_2 and N availability. A meta-analysis of 18 temperate studies found that aboveground biomass increased significantly more in response to elevated CO_2 with higher N availability (de Graaff *et al.*, 2006). On the other hand, Liu *et al.* (2011) did not find a

significant CO_2 by N interaction when the results of four subtropical species were pooled. Only when examining the responses at the species level did they find elevated CO_2 and higher N availability interact to produce significant differences between treatments. While a handful of studies examined how general soil fertility affects plant response to CO_2 (Winter *et al.*, 2001a; 2001b; de Oliveira *et al.*, 2012), experiments investigating the interaction between CO_2 and soil N availability are lacking for tropical species.

We also observed a pattern of a higher liana relative growth and physiological response to CO_2 when P availability was low, but none of the interactions were statistically significant. However, there were an equal number of growth and physiological response variables that showed the opposite: lianas relative response to CO_2 was larger under the high than the low P treatment. We do not find evidence for our hypothesis that lianas would have a reduced relative response to elevated CO_2 under low P availability compared to high P availability.

There were strong and significant main effects for CO_2 and soil nutrient availability in each experiment, even though we found almost no interactions among the treatment variables. In both studies, elevated CO_2 resulted in increased plant growth, biomass, and maximum photosynthetic rate. These results are similar to the main effects of CO_2 observed in Chapter 2 and in many other studies of tropical plant response to CO_2 (see Cernusak *et al.*, 2013 for a review). A larger number of response variables showed significant main effects when the plants were grown with higher soil nutrient availability. Higher soil N availability resulted in plants shifting resources to their leaves, while higher P availability resulted in across the board increases in most growth and biomass variables.

One explanation for the lack of an interaction between CO_2, N, and growth form may be low N availability even in the high N treatment (Table 3.2). Although the concentration of soil

NO_3 at the end of the experiment was more than twice as high in the high N than in the low N treatment, both NO_3 and NH_4 concentrations were quite low compared to ambient soil N concentrations (~14 mg/kg) in nearby forests (Yavitt & Weider, 1988). We expected strong microbial immobilization of N available to plants from the addition of rice husks, yet we added an amount of N equivalent to the original level of the soil to each high N treatment pot on a weekly basis. The fact that N remained low in the high N treatment pots could be explained by a two factors. Plant uptake of N could have been very rapid, depleting the available soil N within a few days after application. We found significant differences between the low and high N treatments that indicate the plants with higher N availability grew faster, produced more leaves, allocated more biomass to leaves, and had a higher maximum photosynthetic rate (Table 3.4). However, we did not observe a significantly lower foliar C:N ratio, indicative of higher foliar N, in the high N treatment. Translocation of N away from the leaves may explain why we failed to detect any increase in N uptake. An alternative explanation for the low N availability in the high N treatment is that microbial immobilization, nitrification, and/or denitrification rapidly depleted plant available N (Dalling *et al.*, 2013). The high C:N ratio of the soil due to the presence of rice husks might have led to such high microbial decomposition rates that any additional inorganic N was quickly immobilized by the microbial community. Similarly, the high organic content of the soil may have led to high nitrification rates with resulting gaseous N losses (Koehler *et al.*, 2009), but we found lower net nitrification rates in the high N compared to the low N treatment (data not shown). Additionally, this experiment was conducted during the wet season where high soil water content may have encouraged high rates of denitrification. While the use of an 80:20 soil to rice husk mixture may have had too strong an N limiting effect, the differences in plant

growth between the N treatments indicate at least some extra N was available for plant uptake in the high N treatment.

The stark difference in the concentration of soil P between lianas and trees in the N experiment (Table 3.2) might at first appear to be the result of higher uptake of soil P by lianas. However, the higher soil P associated with the trees is entirely due to the tree species *Terminalia amazonia*. Average soil P for this species is 14.06 mg kg^{-1} in the low N treatment and 26.03 mg kg^{-1} in the high N treatment, while the soil P for the other tree, *Inga sapindoides*, was comparable to soil P for both liana species (ca. 1.2-1.8 mg kg^{-1}). We do not know whether this was the result of extremely high root phosphatase activity in *Terminalia* because this species was not assayed, but we find it unlikely given that *Inga* had above-average phosphatase activity and yet did not result in higher soil P availability than the three liana species that had correspondingly lower phosphatase activity.

The results of the elevated CO_2 and soil P availability experiment on three liana species provide evidence that soil P may have more of a stimulative effect on liana growth than elevated CO_2. Except for leaf loss, levels of soil P provided neither a constraint nor a boost to liana growth under elevated CO_2 contrary to both our hypothesis and that of Zhu & Cao (2010). Instead, higher soil P alone acted as a stronger growth stimulant than either elevated CO_2 alone or elevated CO_2 in combination with increased soil P. The effect of higher soil P was larger than the effect of elevated CO_2 in both the number of variables with a significant change and the magnitude of that change. We did not include tree species as part of this experiment, and this is the first study to our knowledge to examine tropical plant response to elevated CO_2 and soil P availability, so we do not know whether this effect is stronger for lianas than for trees. However,

we believe this result provides a template for future studies on the underlying mechanisms for the relative increase of lianas over trees.

We have no evidence in our experiments that soil N and P availability interacts with increasing atmospheric CO_2 to significantly affect plant growth and physiology. Instead, changes to soil nutrient availability or CO_2 alone had strong and significant effects on the species studied here. For both the N and P experiments, higher soil nutrient availability led to a larger number of significant differences between treatments than did increasing CO_2. This suggests that while explanations for the reported liana increase may not lie in the interaction among changing biogeochemical cycles, changes within these cycles *alone* could be a potential contributor. We recommend further study of a broader range of liana and tree species for their response to changes in soil N and P availability.

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seasonal rain forest in southwestern China. *Oecologia*, **163**, 591–599.

	Lianas		Trees	
Experiment	Family	Species	Family	Species
Nitrogen	Fabaceae (Faboideae)	*Machaerium milleflorum* Pittier	Combretaceae	*Terminalia amazonia* (J.F. Gmel.) Exell
Nitrogen	Malpighiaceae	*Stigmaphyllon hypargyreum* Triana & Planch.	Fabaceae (Mimosoideae)	*Inga sapindoides* Willd.
Phosphorus	Aristolochiaceae	*Aristolochia anguicida* Jacq.	–	–
Phosphorus	Convolvulaceae	*Bonamia trichantha* Hallier	–	–
Phosphorus	Dilleniaceae	*Davilla nitida* (Vahl) Kubitzki	–	–

Table 3.1 Species used in the nitrogen and phosphorus availability experiments

	Experiment			
	Nitrogen		Phosphorus	
	-N	+N	-P	+P
Nitrate (mg/kg)	**0.12**	**0.28**	**18.3**	**18.8**
L	0.10	0.22	-	-
T	0.13	0.33	-	-
Ammonium (mg/kg)	**0.45**	**0.43**	**0.31**	**0.32**
L	0.45	0.41	-	-
T	0.45	0.44	-	-
Phosphorus (mg/kg)	**4.43**	**7.62**	**0.19**	**7.29**
L	1.53	1.63	-	-
T	7.41	13.06	-	-

Table 3.2. Average soil nutrient availability at the end of the N and P experiments. Bold number indicate the treatment level average. L = pots with liana species; T = pots with tree species; - indicates a treatment combination not used for the study.

| Response Variable | Experiment | |
	Nitrogen	Phosphorus
Growth Change		
Stem Length (cm)	●	●
Diameter (cm)	●	●
Leaf Area (cm^2)	●	●
Total Leaf Production	●	●
Leaf Loss	●	●
Biomass Change		
Leaf Biomass (g)	●	●
Stem Biomass (g)	●	●
Root Biomass (g)	●	●
Total Biomass (g)	●	●
Relative Growth Rate	●	●
Ratios		
Leaf Area Ratio (cm^2 mg^{-1})	●	●
Leaf Mass Area (mg cm^{-2})	●	●
Specific Leaf Area (cm^2 mg^{-1})	●	●
Root:Shoot Ratio	●	●
Leaf:Stem Ratio	●	●
Physiology		
Day: Max Photosynthetic Rate (μmol CO$_2$ m^2 s^{-1})	●	●
Day: Stomatal Conductance (mol H$_2$O m^2 s^{-1})	●	●
Day: Transpiration (mmol H$_2$O m^2 s^{-1})	●	●
Night: Respiration Rate (μmol CO$_2$ m^2 s^{-1})		●
Night: Stomatal Conductance (mol H$_2$O m^2 s^{-1})		●
Night: Transpiration (mmol H$_2$O m^2 s^{-1})		●
Foliar C:N Ratio	●	●
Foliar P (mg/g)		●
δ^{13}C (‰)		●
δ^{15}N (‰)		●
Root Phosphatase (μmol pNP g^{-1} h^{-1})	●[*]	●[†]

[*] Root phosphatase was measured on 5 individuals per treatment (CO$_2$, N) for all species except *Termanalia amazonia* which was not assayed.

[†] Root phosphatase was measured on at least 5 individuals per treatment (CO$_2$, P) in all species except for *Bonamia tricantha* which had insufficient fine roots for the assay under ambient CO$_2$ and ambient P (n=1), and ambient CO$_2$ and reduced P (n=4).

Table 3.3. Variables measured in the experiments and used as the response variables in the model, classified by variable category and experiment.

	Interaction					Main effects						
	Lianas		Trees			CO_2		N		GF		
Response Variable	-N	+N	-N	+N								Random
Growth Change	% Δ_{CO2}	% Δ_{CO2}	% Δ_{CO2}	% Δ_{CO2}	p-val	% Δ	p-val	% Δ	p-val	% Δ	p-val	Structure
Stem Length	47.3	41.2	137.9	-24.9	n.s.	41.4	n.s.	9.3	n.s.	-90.3	n.s.	2
Diameter	93.8	43.7	318.1	11.5	n.s.	**63.1**	**0.007**	39.0	n.s.	-49.6	n.s.	1
Leaf Area	21.5	16.9	30.9	0.6	n.s.	16.2	n.s.	13.5	n.s.	-32.9	n.s.	2
Total Leaf Production	47.1	12.1	82.4	-8.7	n.s.	21.9	n.s.	**34.3**	**0.008**	-11.1	n.s.	2
Leaf Loss	-52.5	-80.0	-34.4	16.8	n.s.	-41.1	n.s.	-34.1	n.s.	75.4	n.s.	2
Biomass Change												
Leaf Biomass	25.0	23.5	20.0	4.5	n.s.	16.1	n.s.	21.4	n.s.	15.4	n.s.	2
Stem Biomass	39.4	31.6	49.8	-12.4	n.s.	23.8	n.s.	0.3	n.s.	-28.8	n.s.	2
Root Biomass	92.1	87.3	54.8	-8.0	n.s.	**46.2**	**0.015**	-8.5	n.s.	-0.3	n.s.	2
Total Biomass	39.6	34.2	33.8	-2.4	n.s.	23.0	n.s.	8.9	n.s.	-3.5	n.s.	2
Relative Growth Rate	34.6	15.5	-26.6	7.2	n.s.	8.5	n.s.	**33.2**	**0.005**	-22.8	n.s.	2
Ratios												
Leaf Area Ratio	0.6	-5.5	-1.2	1.4	n.s.	-1.6	n.s.	**14.6**	**<0.001**	-36.0	n.s.	2
Leaf Mass Area	-3.0	3.2	-3.5	2.3	n.s.	-0.4	n.s.	-0.2	n.s.	**79.0**	**0.017**	2
Specific Leaf Area	1.2	-3.3	-0.8	-1.3	n.s.	-1.0	n.s.	0.0	n.s.	**-43.4**	**0.021**	2
Root:Shoot Ratio	6.0	16.9	-2.6	4.7	n.s.	5.2	n.s.	**-24.9**	**<0.001**	6.4	n.s.	2
Leaf:Stem Ratio	-3.3	26.0	-7.3	7.3	n.s.	4.0	n.s.	**29.1**	**0.007**	**47.9**	**0.006**	2
Physiology												
Max Photosynthetic Rate	30.7	34.2	36.5	24.8	n.s.	**31.5**	**<0.001**	**27.0**	**<0.001**	-7.4	n.s.	1
Stomatal Conductance	18.1	18.2	-33.4	-38.5	n.s.	-10.5	n.s.	17.8	n.s.	-17.8	n.s.	1
Transpiration	5.8	3.0	-29.5	-28.5	n.s.	**-13.2**	**0.047**	12.1	n.s.	-8.5	n.s.	1
Foliar C:N Ratio	12.2	-4.4	4.6	-4.8	n.s.	1.7	n.s.	-4.7	n.s.	18.2	n.s.	1
Root Phosphatase	63.5	25.3	14.6	12.7	n.s.	24.7	n.s.	**27.1**	**0.04**	**184.1**	**<0.001**	2

Table 3.4. Mixed model estimates of liana and tree % response to CO_2 treatment and p-values for growth, biomass, response ratio, and physiological variables in the N experiment. Results of the three-way interaction between growth form, CO_2 treatment, and N treatment are on the left side of the table, while main effects of each treatment are on the right. These values take into account the environmental covariates and random effects used in the model. The random effects structure is in the far right-hand column. $1 = (\beta_{Chamber(i)} + \gamma_{Species(i)})$; $2 = (\gamma_{Species(i)})$

| Response Variable **Growth Change**	Interaction			Main Effects				Random Structure
	-P % Δ_{CO2}	+P % Δ_{CO2}	p-val	CO$_2$ % Δ	p-val	P % Δ	p-val	
Stem Height	26.3	12.7	n.s.	20.2	n.s.	**33.0**	**0.048**	2
Diameter	58.5	-11.6	n.s.	15.8	n.s.	32.2	n.s.	2
Leaf Area	33.5	39.7	n.s.	**39.4**	**0.031**	**43.5**	**0.018**	2
Total Leaf Production	33.7	6.5	n.s.	18.6	n.s.	**32.5**	**0.034**	1
Leaf Loss	**120.7**	**-92.2**	**0.013**	-17.2	n.s.	-39.5	n.s.	2
Biomass Change								
Leaf Biomass	31.8	42.0	n.s.	**40.2**	**0.039**	**47.2**	**0.018**	2
Stem Biomass	28.4	41.4	n.s.	38.0	n.s.	**42.3**	**0.042**	2
Root Biomass	-2.5	11.3	n.s.	5.9	n.s.	23.5	n.s.	2
Total Biomass	17.7	37.4	n.s.	30.8	n.s.	**42.3**	**0.014**	2
Relative Growth Rate	56.1	14.2	n.s.	31.2	n.s.	**53.4**	**0.004**	1
Ratios								
Leaf Area Ratio	16.5	13.3	n.s.	**15.9**	**0.033**	**21.5**	**0.005**	2
Leaf Mass Area	-1.8	5.3	n.s.	0.4	n.s.	**-16.4**	**0.004**	2
Specific Leaf Area	15.8	2.3	n.s.	9.3	n.s.	**14.6**	**0.007**	2
Root:Shoot Ratio	-35.2	-22.0	n.s.	**-30.1**	**0.017**	-16.5	n.s.	2
Leaf:Stem Ratio	-6.9	5.6	n.s.	0.9	n.s.	**24.9**	**0.01**	2
Physiology								
Max Photosynthetic Rate	38.9	56.8	n.s.	**49.1**	**<0.001**	**23.7**	**0.022**	1
Stomatal Conductance	-24.2	6.5	n.s.	-9.8	n.s.	7.1	n.s.	2
Transpiration	-18.0	12.4	n.s.	-4.1	n.s.	4.1	n.s.	2
Night: Respiration Rate	-46.4	-7.7	n.s.	-35.2	n.s.	-38.6	n.s.	2
Night: Stomatal Conductance	-12.7	-0.8	n.s.	-7.8	n.s.	-14.3	n.s.	1
Night: Transpiration	-9.7	1.9	n.s.	-5.1	n.s.	-16.5	n.s.	1
Foliar C:N Ratio	14.1	-9.5	n.s.	2.2	n.s.	**-11.6**	**0.046**	2
Foliar P	-11.7	1.5	n.s.	-4.3	n.s.	**24.5**	**<0.001**	2
δC13	3.2	2.9	n.s.	**3.1**	**<0.001**	0.2	n.s.	2
δN15	4.0	-8.4	n.s.	-2.4	n.s.	**-21.0**	**<0.001**	2
Root Phosphatase	-5.9	-2.4	n.s.	-5.4	n.s.	-10.1	n.s.	2

Table 3.5. Mixed model estimates of liana % response to CO$_2$ treatment and p-values for growth, biomass, response ratio, and physiological variables in the P experiment. Results of the two-way interaction between CO$_2$ treatment and P treatment are on the left side of the table, while main effects of each treatment are on the right. These values take into account the environmental covariates and random effects used in the model. The random effects structure is in the far right-hand column. $1 = (\beta_{Chamber(i)} + \gamma_{Species(i)})$; $2 = (\gamma_{Species(i)})$
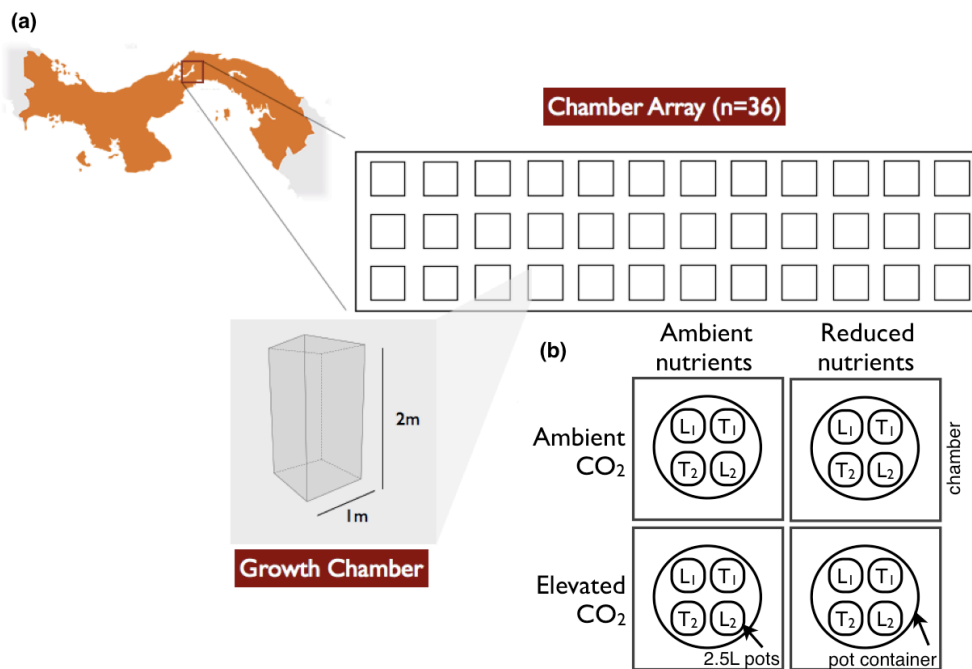
Figure 3.1. a) Open top chamber array location, layout, and dimensions b) Experimental design and species distribution among treatments and within chambers, L = liana, T = tree; each subscript number represents a distinct species (Note: three liana species and no tree species were used in the P experiment). Species locations within each chamber for both experiments were randomized.
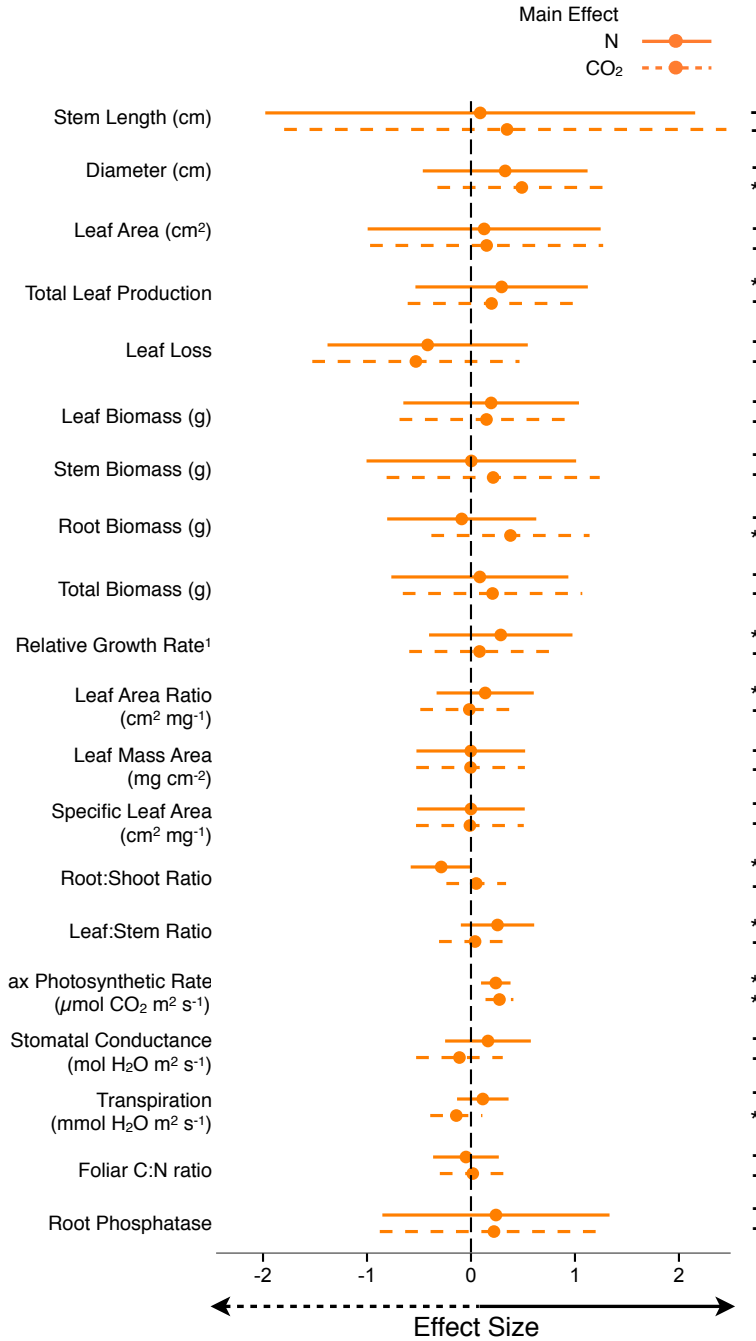
Figure 3.2. Effect size of main effect response to N treatment (solid lines) and CO_2 treatment (dashed lines) for all response variables. Positive/negative effect sizes indicate an increased/decreased response to the treatment. Points represent the mean effect size, lines represent the 95% confidence interval. * indicate significant differences in treatment effect (P<0.05), while – indicates no significant difference in treatment effect.
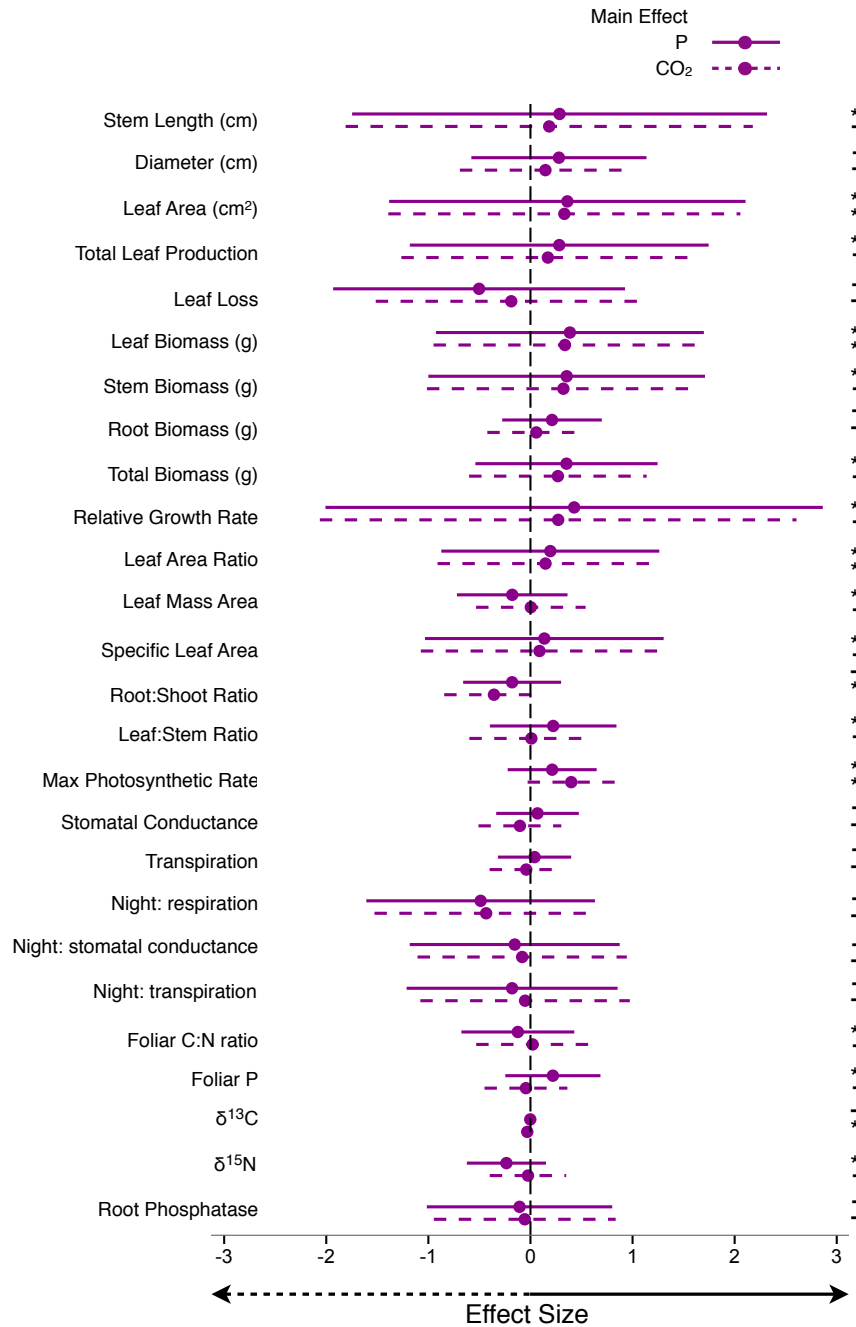
Figure 3.3. Effect size of main effect response to P treatment (solid lines) and CO_2 treatment (dashed lines) for all response variables. Positive/negative effect sizes indicate an increased/decreased response to the treatment. Points represent the mean effect size, lines represent the 95% confidence interval. * indicate significant differences in treatment effect ($P<0.05$), while – indicates no significant difference in treatment effect.

Chapter IV

Mapping liana canopy cover across tropical forest landscapes using high-resolution imaging spectroscopy[*]

Abstract

Increasing size and abundance of lianas relative to trees are among the pervasive changes observed in undisturbed neotropical forests over the last two decades. The negative effect that lianas exert on tree growth, reproduction, and lifespan, combined with their very low contribution to forest biomass, suggest a future in which neotropical forests will absorb and store less atmospheric carbon dioxide annually. Yet the liana growth form is chronically understudied in forest censuses, resulting in few data on the scale, cause, and impact of increasing lianas. Satellite and airborne remote sensing provide ecologists with the tools that potentially can map and monitor lianas at very large spatial and rapid temporal scales, compared with plot-based forest censuses. Contrasting foliar chemical and structural properties between lianas and trees result in documented differences in the reflectance spectra of the growth forms. Recent advances in imaging spectrometers and classification algorithms provide the possibility of distinguishing levels of liana coverage at the tree canopy scale. We combined high-resolution airborne imaging spectroscopy and a ground-based tree canopy census of an intact, seasonally dry forest in central Panama to investigate whether tree canopies supporting lianas could be discriminated from tree

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canopies with no liana coverage. Using support vector machine classification algorithms, we achieved testing accuracies of greater than 90% in discriminating trees with severe (>80%) liana canopy cover from trees with 0% liana cover. When applied to the full image of the study site, the classification model had a 2.3% false-positive error rate when validated against an independent plot-level dataset of liana canopy cover. Using our landscape-scale liana cover classification map, we show that 11.9%-18.0% of the 585 ha study site has >80% liana canopy cover. When viewed in the context of the relative increase in lianas, this extent of severe liana canopy cover across such a large fraction of the landscape has broad implications for ecosystem function and forest carbon storage.

Introduction

Tropical forests are a critical part of the global climate system and carbon cycle. Intact tropical forests alone absorb and store c. 1.19 ± 0.41 Pg carbon yr^{-1} from the atmosphere (Pan *et al.*, 2011), an amount equivalent to 12.3% of total global carbon emissions in 2012 (Peters *et al.*, 2013). For perspective, this amount is greater than all yearly carbon emissions from the European Union (CDIAC, 2012). Recently, plot-based studies in the neotropics have documented pervasive changes in old-growth forests that may alter their role in the global carbon cycle. These changes include increased biomass and productivity (Phillips *et al.*, 1998; Baker *et al.*, 2004), increased tree turnover (Phillips *et al.*, 2004), and shifted floristic composition (Korner, 2004; Laurance *et al.*, 2004; Feeley *et al.*, 2011; Schnitzer & Bongers, 2011).

Tropical lianas (woody vines) are reported to be increasing relative to trees in neotropical forests over recent decades (Schnitzer & Bongers, 2011; Schnitzer *et al.*, 2012; Yorke *et al.*, 2013). Reported annual increases in liana stem abundance range from 0.23% to 7.8%, while in the same study areas trees either underwent smaller annual increases or have declined in stem

abundance (Phillips *et al.*, 2002; Chave *et al.*, 2008; Schnitzer *et al.*, 2012; Yorke *et al.*, 2013).

These same studies found increases in liana basal area ranging from 0.6% to 4.6% annually over

the same time period, with just a 0.34% per year increase in tree basal area. Liana seedling

recruitment, reproduction, leaf productivity, and canopy cover have also increased relative to

trees (Benítez-Malvido & Martinez-Ramos, 2003; Wright *et al.*, 2004; Wright & Calderon, 2006;

Wright *et al.*, 2008; Ingwell *et al.*, 2010).

The reported relative increase of lianas has broad implications for tropical forests and the

global carbon cycle. Lianas commonly comprise a large proportion (see Chapter 1) of the woody

species and stem numbers in tropical forests (Schnitzer *et al*., 2012); however, lianas constitute

only a small proportion of total tropical forest biomass (Putz, 1983; Gerwing & Farias, 2000;

DeWalt & Chave, 2004; Letcher & Chazdon, 2009; Durán & Gianoli, 2013). Nevertheless, lianas

have a disproportionately large negative effect on tree biomass accumulation by reducing tree

diameter increment (Lowe & Walker, 1977; Whigham, 1984; Clark & Clark, 1990; Grauel &

Putz, 2004; van der Heijden & Phillips, 2009; Schnitzer *et al*., unpublished data), leaf

productivity (Dillenburg *et al.*, 1993; Perez-Salicrup *et al.*, 2001; Toledo-Aceves & Swaine,

2008), sap flow velocity (Tobin *et al.*, 2012; Alvarez-Cansino *et al*., unpublished data), and stem

height (Perez-Salicrup, 2001). Lianas also decrease forest carbon accumulation and long-term

storage through reduced tree fecundity (Stevens, 1987; Kainer *et al.*, 2006; Nabe-Nielsen *et al.*,

2009), increased tree mortality (Putz, 1984; Phillips *et al.*, 2002; Garrido-Perez *et al.*, 2008;

Ingwell *et al.*, 2010; Schnitzer *et al*., unpublished data), and suppressed tree regeneration

(Toledo-Aceves & Swaine, 2008; Schnitzer & Carson, 2010). Trees that support large lianas or

severe liana infestations have a 40-100% increased mortality risk (Phillips *et al.*, 2002; Ingwell *et

al.*, 2010). The disproportionately negative effect that lianas can exert on tree growth,

reproduction, and lifespan, combined with their low contribution (Durán & Gianoli, 2013) to forest biomass, suggest a future in which neotropical forests will absorb and store less atmospheric carbon dioxide annually (van der Heijen *et al.*, 2013).

Despite the negative consequences of increasing lianas, few studies have examined temporal changes in tropical liana abundance and size. While some studies rely on proxy data (i.e., flowering, productivity, recruitment) to establish that lianas are increasing relative to trees, only five studies have used stem or canopy-based censuses (Phillips *et al.*, 2002; Chave *et al.*, 2008; Ingwell *et al.*, 2011; Schnitzer *et al.*, 2012; Yorke *et al.*, 2013). These studies examine a total of 125 ha of neotropical old-growth tropical forests among 114 plots ranging in size from 0.1 ha to 50 ha (Figure 4.1). The limited spatial extent of long-term liana censuses restricts our ability to assess the scale and impact of increasing tropical lianas.

Satellite and airborne remote sensing may allow ecologists to map and monitor liana abundance at far larger spatial, and more rapid temporal, scales than plot-based censuses. Previous lab and field studies have documented clear differences between liana and tree spectral reflectance signatures, with supporting foliar chemical and structural data (Supplementary Information, Table S4.1). These studies document specific regions of the electromagnetic spectrum where lianas and trees are separable, and show that leaf-level differences scale up to the canopy level. Only one study has successfully used remote sensing to map liana abundance at the landscape-scale. This study successfully identified 1150 ha of forest with severe liana canopy cover using moderate-resolution hyperspectral and multispectral imagery (Foster *et al.*, 2008). However, the liana patches mapped in this study were within large (>0.45 ha) forest gaps with severe (c. >80%) liana cover, thus allowing detection with moderate-resolution imagery. It is

unlikely the same approach could successfully map liana abundance and distribution in contiguous closed-canopy neotropical forests.

Recent advances in high-resolution imaging spectroscopy technology and analysis techniques now provide the potential to distinguish lianas from trees at the sub-canopy scale. Next generation high spatial and spectral resolution imaging spectrometers have the capability to discriminate subtle differences in leaf chemistry and structure (Kampe *et al.*, 2010; Asner & Martin, 2010), and new supervised classification methods have proven accurate at discriminating individual tree species (Colgan *et al.*, 2012; Féret & Asner, 2012).

Our goal was to map the distribution and abundance of lianas in a seasonally dry neotropical forest using high-resolution imaging spectroscopy. Using imagery collected over central Panama by the Carnegie Airborne Observatory, combined with a ground-based liana canopy census of nearly 800 trees, we asked whether liana canopy cover could be mapped over a 600 ha contiguous closed-canopy neotropical forest. We employed support vector machine classification algorithms, and evaluated their ability to discriminate between trees and lianas. We also explored the lower threshold of liana canopy cover for which liana-supporting trees could be accurately distinguished from liana-free trees.

Methods

Site

The study site is a mainland peninsula of the Barro Colorado Nature Monument in the Republic of Panama (Figure 4.2). The Gigante Peninsula (9.1°N, 79.8°W) is covered by a seasonally-dry, secondary tropical moist forest >200 years old, interspersed with 50-70 year old forest patches recovering from agricultural disturbance (D. Dent unpublished data). The

geological substrate is a Miocene basalt (Stewart *et al*., 1980), and the soils are considered

relatively fertile for the lowland tropics (Wright *et al.*, 2011). On nearby Barro Colorado Island,

monthly precipitation averages c. 290 mm in the wet season (May-December) and c. 70 mm in

the dry season (January-April; STRI, 2013).

Imagery

In February of 2012, the Carnegie Airborne Observatory (CAO) Airborne Taxonomic

Mapping System (AToMS) acquired high-resolution data of the site with an integrated (i) full-

spectral range imaging spectrometer, (ii) a zoom imaging spectrometer, and (iii) a full-waveform

LiDAR. Details of the AToMS are described in Asner *et al.* (2012). The visible-to-shortwave

infrared (VSWIR) imaging spectrometer collects data in 428 contiguous spectral bands from

380-2510 nm, with a signal-to-noise ratio that is up to five times higher than NASA's Airborne

Visible and Near-infrared Imaging Spectrometer (AVIRIS). Deployment of the CAO at a flight

altitude of 2000 m resulted in imagery with a 2 m pixel spatial resolution. The visible-to-near

infrared (VNIR) zoom imaging spectrometer collects 288 contiguous spectral bands over a

smaller range (365-1052 nm) than the VSWIR, but at twice the pixel spatial resolution (1.0 m at

2000 m altitude).

We used LiDAR pulses that reached the ground surface to interpolate a raster digital

terrain model (DTM) for the ground surface. This was achieved using a 10 m × 10 m kernel

passed over each flight block; the lowest elevation estimate in each kernel was assumed to be

ground. Subsequent points were evaluated by fitting a horizontal plane to each of the ground seed

points. If the closest unclassified point was < 5.5° and < 1.5 m higher in elevation, it was

classified as ground. This process was repeated until all points within the block were evaluated.

We radiometrically corrected the VSWIR data to radiance (W sr^{-1} m^{-2}) using a flat-field correction, radiometric calibration coefficients, and spectral calibration data collected in the laboratory. We created a camera model to precisely describe the three-dimensional location and field-of view of each sensor and, combined with standardized timing information, for high-precision data co-registration. We then used a smoothed best estimate of trajectory (SBET), LiDAR DTM, and camera model to produce an image geometry model and observational data containing information on exact solar and viewing geometry for each image pixel. We used these inputs to atmospherically correct the radiance imagery to apparent surface reflectance using the ACORN-5 model (Imspec LLC, Glendale, CA USA). To improve aerosol corrections in ACORN-5, we iteratively ran the model with different visibilities until the reflectance at 420 nm (which is almost constant for vegetated pixels) was 1%. We then corrected the reflectance data for cross-track brightness gradients using a bidirectional reflectance distribution function (BRDF) model (Colgan *et al.*, 2012). Finally we geo-orthorectified the imagery to the LiDAR DTM, with accuracy better than 10 cm based on an embedded Global Navigation Satellite System (GNSS) and Inertial Measurement Unit (IMU).

We used a 1270 by 1800 pixel VSWIR image of the study site covering 600 ha. We removed water absorption bands and bands near the instrument measurement boundaries, resulting in a 178-band VSWIR image used for the analyses described in "Support Vector Machine Classification" below. In addition, the precise positioning of the lidar-to-VSWIR data allowed for automated masking of pixels shaded by neighboring canopies and branches, as well as water bodies (Asner *et al*., 2007). We used a 4-band subset VNIR image of the same area only to identify and georeference individual tree crowns in the field (see "Field Data" below).

Field Data

Individual tree crown georeferencing: During July and August 2013, we collected field data outside the boundaries of ongoing forest manipulation experiments at the study site. We used a combined tablet computer (Apple Inc., Cupertino, CA USA) and Bluetooth-enabled GPS/GLONASS receiver (Garmin Ltd., Olathe, KS USA) system to navigate and collect field data within the study site. We uploaded the VNIR image of the study site to the application iGIS (Geometry Pty Ltd., Tasmania, Australia) on the tablet system, allowing us to georeference individual tree crowns directly on the imagery. Once an individual tree crown in the image was confirmed on the ground, we marked the tree in the iGIS application and recorded all data in a custom data entry pop-up form linked to each point. We only marked trees ≥10 cm diameter at breast height (dbh) that had 90% of the crown fully sun-exposed and were clearly identifiable on the imagery (n=775). All point coordinates and associated data were exported from the tablet system as shapefiles.

Liana canopy cover survey: We assessed the percent cover of lianas in each georeferenced tree canopy using an improved version of the crown occupation index (cf. Clark & Clark, 1990; van der Heijden *et al.*, 2010), as follows. The field team consisted of four people working in pairs, which rotated membership each day. In our improved version of the index, the centroid of each tree's canopy was first determined, and the crown then visually bisected with north-south and east-west lines, forming four quadrants. Independent of their partner, each person thoroughly assessed each quadrant for the percent cover of lianas to the nearest 5%. The two partners then discussed the quadrant estimates and mutually agreed on a final estimate for each. To assess inter-rater reliability, at the beginning of each field day one tree was independently assessed by each of the four team members before splitting into pairs. We also measured the dbh and made qualitative estimates of the leaf area index (LAI) of each tree, noting

any major crown gaps or irregularities. We recorded the species identifications of commonly occurring trees only.

   Individual tree crown pixel extraction: Using the VNIR image in ERDAS IMAGINE (Hexagon Intergraph, Madison, AL USA) or ENVI (Exelis, Boulder, CO USA) software, we outlined the sunlit portions of the crown for each georeferenced tree, carefully avoiding shaded areas and crown edges. We extracted pixels from the VSWIR image using the crown polygons that encompassed at least three image pixels (smaller crowns were excluded). We calculated the normalized difference vegetation index (NDVI) as (NIR – VIS)/(NIR + VIS) where NIR and VIS are reflectances at 800 and 680nm, respectively. We filtered the data to retain only well-lit, live vegetation pixels with an NDVI ≥0.8 and mean near infrared (850-1050 nm) reflectance >20%. This yielded a total of 607 usable tree crowns in the analysis, representing a total of 23,270 pixels. The distribution of individual tree crown liana canopy cover is presented in Figure 4.3.

Support Vector Machine Classification

   Support vector machine (SVM) is a supervised machine learning technique increasing in use among the remote sensing community (Mountrakis *et al.*, 2011). We chose to use SVM because they produce comparable or better results than other classification algorithms such as discriminant analysis, maximum likelihood, or artificial neural networks (Mountrakis *et al.*, 2011). SVM is a non-parametric classifier in which no assumptions about the underlying distribution of the data are made, making SVM particularly useful in remote sensing applications where the imagery data tend to have unknown distributions. SVM projects samples of different classes into multidimensional space and fits a hyperplane that best defines the boundaries separating the classes. To transform the hyperspectral data into higher dimensional space, we used the radial basis function (RBF) kernel because it has a low number of input parameters and

higher performance relative to other kernel functions (Féret & Asner, 2012). Other advantages of SVM are the ability to efficiently process large input spaces and its insensitivity to the Hughes phenomenon, or the decrease in classification accuracy after passing a threshold number of input features (Melgani & Bruzzone, 2004). This allows full use of the high dimensionality of hyperspectral data with relatively few training samples (Gualtieri & Cromp 1999).

The general framework of SVM classification is similar to other classification techniques. First, data are split into training (SVM fitting) and testing (SVM validation) sets of a given proportion and balance (i.e., between-class proportion). Since we are evaluating liana cover of individual tree crowns, we selected pixels for the testing and training sets at the crown level, such that all pixels were included from a selected crown. Second, two SVM model parameters specific to the RBF kernel are optimized. Third, the performance and sensitivity of the SVM to the training/testing set proportion and balance are evaluated. Fourth, the final model is developed, validated, and applied to the entire image under investigation for the purpose of mapping liana coverage classes. All data processing and analysis was performed in the open-source statistical software program R (R Development Core Team, 2012). SVM implementation was performed using the R package 'e1071' (Meyer *et al*. 2012).

SVM Classification, Optimization, and Validation

We binned all trees crowns from the field survey into six liana canopy cover classes and assigned this value to all pixels of each crown: zero (0% cover), thin (1-20% cover), mild (21-40% cover), moderate (41-60% cover), heavy (61-80% cover), and severe (81-100% cover). We separately tested five different threshold values (>80%, >60%, >40%, >20%, ≥1%) against the 0% liana cover class. These binary threshold classifications were used to evaluate the percentage canopy cover at which lianas can be accurately detected.

Before the final SVM classification and validation can be performed, the model

parameters are optimized. The first parameter to be optimized for the RBF kernel is the gamma

(ϒ) parameter controlling the flexibility of the classifier – or the trade off between model over-

fitting and under-fitting (Ben-Hur & Weston, 2009). The second parameter, penalty or cost (C),

controls the trade off between model complexity and training errors (Cortes & Vapnik, 1995).

We used an exhaustive grid search of the parameter space from $1e^{-10}$ to $1e^{10}$ for both, selecting

the global optimum of the ϒ and C parameters based on the results of a 5-fold cross-validation of

the model. This was done for each binary threshold classification, and the respective parameter

set was used for all future SVM testing and implementation.

We also tested SVM performance and sensitivity to the size and balance of sample

training data. For each binary threshold classification, we input varying proportions of each class

as training data to the SVM and evaluated the model performance when applied to the testing

dataset (i.e., remaining tree crowns not selected for the training data). For each training data

combination, we repeated this 100 times with a randomly selected sample of tree crowns, and

computed the mean pixel-level testing classification accuracy and standard deviation. The

unbalanced nature of the testing data (Figure 4.3) would strongly bias the reported accuracies in

favor of the 0% liana cover in tree crowns. Therefore we calculated the pixel-level testing dataset

balanced accuracy (BAC) following Féret & Asner (2012) as:

$$\mathrm{BAC} = \frac{\mathrm{P(target) + P(non-target)}}{2} \times 100$$

where P(target) is the proportion of pixels correctly classified in the target class (i.e., tree crowns

with liana cover) and P(non-target) is the proportion of pixels correctly classified in the non-

target class (i.e., tree crowns without liana cover). From the 100 SVM model iterations, we also

computed the mean crown-level testing classification BAC and standard deviation, whereby the

classified pixels are averaged over each tree crown, and the crown subsequently assigned to the liana cover class with the highest proportion of pixels.

For each binary threshold classification we selected the training dataset combination that produced the highest mean BAC at the pixel and crown levels. Then from among the 100 SVM model iterations within each of the best training dataset combinations we chose the SVM classification model based on the testing dataset that achieved the highest BAC. The binary threshold classification with the highest resulting SVM model BAC was used to map liana canopy coverage (see "Landscape liana cover mapping"), and this final model is referred to as the "optimal SVM."

Strong cross-track brightness gradients within each flight line in the mosaicked image are present because of the time of day the site was flown by the CAO (1:00-2:30pm). We created a subset of the original VSWIR image that included only sections of each flight line closest to nadir that showed the least cross-track brightness gradient. Using the same procedures described above, we constructed a separate optimal SVM based on tree crowns extracted from just the VSWIR image subset with uniform brightness, and is referred to as the "optimal subset SVM." The results from this procedure were used to assess the effect of flight line bias in liana cover classification only.

Landscape Liana Cover Mapping

We applied the optimal SVM model to the full VSWIR image extent of the study site. We assessed the total liana coverage of the study site from this classified image. We applied a 5m inland buffer around all water features to remove any influence of below-canopy water reflectance. We summed the pixels in each class over the whole image to calculate the percent coverage for each class.

We performed the same procedure with the optimal subset SVM. We assessed the effects of the cross track brightness gradient by comparing the distributions of classified pixels from the optimal subset SVM and those pixels classified by the optimal SVM in the same image subset. The results from this procedure were used to assess the effect of flight line bias in liana cover classification only.

To validate the full landscape liana cover map, we used data from an ongoing liana removal experiment at the study site (Schnitzer *et al*., unpublished data). Sixteen 80 by 80 m plots have been censused for all lianas and trees >1cm dbh. In April 2011, all lianas were cut near the soil surface from eight of the plots, and new liana sprouts have been pruned every 3 months. The other eight plots are unmanipulated controls, and a similar liana canopy cover assessment as described in "Liana canopy cover survey" above is conducted every 6 months by their team. We calculated the number of pixels incorrectly classified by the SVM as lianas in the removal plots. We also compared the number of liana-classified pixels in each control plot to the total plot-level liana canopy cover as determined by the canopy survey. Only trees that were closest to the severe liana canopy cover survey class (>75% liana cover) and had sun-exposed crowns were included in these calculations.

Results

SVM Classification Performance

All of the binary threshold classifications achieved >90% cross-validation accuracy on the training data, but the severe liana cover classification outperformed the others with a mean cross-validation accuracy of 96.5±0.6% (Table 4.1). The severe cover classification had the best pixel-level mean BAC of 78.3%±5.8%, although the heavy liana cover classification was not far

behind with 73.1%±1.6% (Figure 4.5; Table 4.1). Crown-level mean BAC was similar between the severe and heavy cover classifications (75.8±9.4% and 76.1±15.8%, respectively). While the moderate threshold classification did not achieve the same level of accuracy as the severe and heavy threshold classifications, it nonetheless performed well with a BAC of 68.3±4.0% (pixel-level) and 66.3±5.9% (crown-level). The mild and thin binary threshold classifications did not achieve mean BAC values that were as high as the other threshold classifications for either the pixel- or crown-level assessments (Figure 4.5; Table 4.1). This indicates that trees with thin to mild (i.e., 1-40%) liana canopy loads are unlikely to be separable from trees without any lianas present. Similar results were found when using data selected only for the optimal subset SVM (not shown).

From among all of the binary threshold classifications, the severe threshold classification had the SVM model with the highest pixel-level (94.2%) and crown-level (92.4%) BAC (Table 4.1). The heavy threshold classification followed with a pixel-level and crown-level BAC of 83.6% and 79.2%, respectively. The best pixel-level moderate threshold classification achieved a moderate BAC of 73.8%, but the crown-level BAC dropped to 68.4%. The best SVM models of the mild and thin threshold classifications did not perform well (Table 4.1).

Given the high performance of the severe threshold classification (94.2% pixel-level BAC), we used this SVM to produce liana cover classifications for the landscape-scale liana cover maps. Using crowns from the full image, the optimal SVM model was a model consisting of 98 tree crowns totaling 5640 pixels with no liana cover (30% of total within-class pixels) and 216 pixels with >80% liana cover (80% of total within-class pixels). This model had a 97.4% cross-validation accuracy of the training data.

When using crowns only from the data selected only for the optimal subset SVM, a slightly better model was produced. The BAC of this optimal subset SVM model was 96.8% (pixel-level), with a 97.3% model cross-validation accuracy of the training data.

Landscape Liana Cover Mapping

The optimal SVM model applied to the full extent of the VSWIR image resulted in a total classified area of 585 ha (Figure 4.6). Pixels in the image were classified as 59.9% trees with 0% liana cover and 18.0% trees with severe liana cover (>80%). A large percentage (22.1%) of the image was not classified because it contained pixels that were shaded or contained dead and/or deciduous vegetation.

The optimal subset SVM model was applied to an image subset of 264 ha (not shown). Pixels in this image subset were classified as 61.6% trees with 0% liana cover and 15.7% trees with severe liana cover (>80%). A similar percentage of unclassified shaded/dead/deciduous pixels were present (22.7%). When the optimal SVM model is applied to the same image subset, 22.7% of the pixels are unclassified, 58.6% are classified as 0% liana cover tree pixels, and 19.0% are classified as severe liana cover pixels. The difference in the percent of pixels classified as trees with severe liana cover using the optimal subset SVM vs. the optimal SVM indicates that there is a small bias (3.3%) toward overclassification of crowns with severe liana infestation due to differences in the non-uniform cross-track brightness of the full image.

Examining the pixel classification of the liana experimental removal plots, we find that the optimal SVM model incorrectly classified an average of only $2.8 \pm 4.2\%$ pixels as containing severe liana cover per removal plot. In the unmanipulated control plots, an average of $16.5 \pm 8.5\%$ of the pixels are classified as severe liana cover. The plot-level pixel classifications differed significantly between the removal and control plots (Mann-Whitney U-test, W=60, p=0.004).

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When we compare the average liana classified pixels in the control plots (16.5±8.5%) to the surveyed percent liana cover of those same plots (22.0±10.3%), no significant difference is detected (Mann-Whitney U-test, W=24, p=0.442). Both the low false positive error rate (2.8%±4.2%) in the removal plots and the similarity between classified and surveyed liana cover in the control plots support the validity of the optimal SVM model.

Incorporating the severe liana cover overclassification bias due to non-uniform cross-track brightness (3.3%) and the false positive error rate (2.8%), we estimate a landscape-scale presence of severe liana cover in the range of 11.9%-18.0%.

Discussion

We developed a method to successfully map the distribution of severe (>80%) liana canopy coverage at the landscape scale in a contiguous tropical forest. Of the 585 ha we classified in the study site, 11.9%-18.0% (70-105 ha) were identified as containing tree canopies with severe levels of liana coverage. Ingwell *et al.* (2011) reported that 16.0% of the 2127 tree crowns they surveyed on nearby Barro Colorado Island had a liana canopy cover > 75%. Our landscape classification estimate of this highest liana cover class is not very different from the average of the surveyed experimental control plots (22.0%) at the study site. Thus, our estimate of severe liana coverage from the SVM classification is within the range of reported values in the area, at least at the plot-level.

An association between the topography of the site and the presence of severe liana canopy cover was detected. Severe liana canopy cover is concentrated along the central plateau, ridgetops, and in valley areas more than on slopes (Figure 4.6c). Stable topographic positions such as plateaus and ridgetops tend to contain nutrient depleted soils as rainfall runoff carries available nutrients downslope (Silver *et al.*, 1994; Vitousek *et al.*, 2003). Plateaus and ridgetops

also tend to have lower soil water availability than slopes (Daws *et al.*, 2002). The low water and nutrient availability of plateaus and ridges may give lianas an advantage over trees given the higher-water use efficiency, wider vessel elements, and potentially deeper roots of lianas (Schnitzer, 2005; Foster & Brooks, 2005; Domingues *et al.*, 2007; Cai *et al.*, 2009), combined with their higher nitrogen- and phosphorus-use efficiencies (Zhu & Cao, 2010). The combination of these properties may lead to local dominance of lianas over trees at stable topographic positions. As erosional and depositional processes increase soil nutrient availability on slopes and valleys, respectively, we should expect the liana advantage to decrease along with liana canopy abundance.

However, we also found severe levels of liana canopy cover at lower elevations and in some of the riparian valleys (lower middle-left portion of Figure 4.6a,c). Some of this area shows flight line and image mosaicking artifacts, evidenced by the high concentration of liana-classified pixels that abruptly end to form a diagonal stripe in this portion of the image. However, not all of the severe cover of lianas in this area is an artifact, as we found large patches (>1ha) of low-lying, liana-dominated forests in this portion of the study site during our field work. This may be the result of recent (~50-70 years ago) agricultural disturbance throughout this portion of the study site (D. Dent, unpublished data), rather than the influence of soil water and nutrient availability on liana-tree competitive interactions.

Although we did not use binary threshold classifications other than the severe cover threshold to classify liana abundance at the landscape scale, the BAC of the heavy and moderate thresholds were comparable to that of the severe threshold classification (Figure 4.5; Table 4.1). This suggests that the lower boundary for accurate liana detection may be as low as 40% canopy coverage. This is supported by results from a study by Kalacska *et al.* (2007) which found low

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testing error rates (~14%) in discriminating trees crowns with liana coverage >40% from tree crowns with no lianas. Further investigation into the lower threshold of liana detection using SVM models is needed.

The widespread severe liana coverage in tree canopies detected at the plot and landscape scales has significant implications for forest carbon dynamics, especially in the context of the reported neotropical liana size and abundance increase relative to trees. Liana loadings are associated with reductions in the carbon gain of trees at the stand level by an average of 0.25 Mg C ha^{-1} y^{-1} (van der Heijden & Phillips 2009). While these authors did not estimate the liana canopy coverage of the trees measured, they did find that as the basal area of lianas entering tree canopies increased, the growth rates of those trees strongly decreased. In fact, Ingwell *et al.* (2011) found that the mortality rate of trees with ≥75% liana canopy cover was double that of trees supporting fewer lianas. If severe liana canopy cover is between 11.9% and 18% of a forested landscape, and also increasing annually, the impact on forest carbon storage could be substantial.

Before we can begin extracting estimated landscape liana coverage from other forests across the neotropics, further refinement of the methods presented are needed. Data reduction techniques to remove the influence of flight line and image mosaicking are important. Principle components (PC) transformation of the input bands produces orthogonal (i.e., uncorrelated) output bands that can be visually examined to discard those PC bands most associated with flight line, mosaicking artifacts, or other noise (Asner *et al.*, 2012). The remaining bands can then be fit to an SVM model for classification. The imbalance between liana-free tree crowns and tree crowns with heavy to severe liana canopy cover highlights the need to target field data collection

to include a higher proportion of heavy to severe liana cover trees. Both of these steps should improve the accuracy and validity of the SVM classification models investigated here.

This study was performed on imagery collected from a seasonally dry forest during the dry season. Previous work in nearby forests in Panama have shown that the reflectance spectra of lianas and trees are most different in seasonally dry forests (Castro-Esau *et al.*, 2004; Sanchez-Azofeifa *et al.*, 2009) and during the dry season (Hesketh & Sánchez-Azofeifa, 2012), but tend to converge in aseasonal forests and during the wet season. Whether the methods presented here would achieve similar detection accuracies during the wet season at this site or in aseasonal forests needs to be explored.

By combining ground-based canopy censuses with high-resolution imaging spectroscopy and machine learning classification algorithms, we have demonstrated the potential of mapping liana abundance at the landscape scale in neotropical forests. The refinement and deployment of these tools will be critical in verifying, quantifying, and monitoring the increase of lianas relative to trees across the neotropics. By uncovering the scale and velocity of the liana increase can we truly begin to understand what impact it will have on the role of tropical forests in the global climate system and carbon cycle.

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| Threshold classification		severe		heavy		moderate		mild		thin	
Liana cover classes		0%	>80%	0%	>60%	0%	>40%	0%	>20%	0%	≥1%
Proportion training (n)		0.15 (38)	0.8 (22)	0.2 (51)	0.76 (38)	0.2 (51)	0.4 (47)	0.2 (51)	0.2 (39)	0.25 (64)	0.25 (88)
		SVM cross validation	Balanced accuracy	SVM cross validation	Balanced accuracy	SVM cross validation	Balanced accuracy	SVM cross validation	Balanced accuracy	SVM cross validation	Balanced accuracy
pixel-level	mean (SD)	96.5 (0.6)	78.3 (5.8)	94.1 (1.0)	73.1 (1.6)	92.8 (1.0)	68.3 (4.0)	92.4 (1.2)	62.4 (1.3)	93.2 (0.7)	56.5 (18.3)
	best SVM	97.4	94.2	92.7	83.6	92.7	73.8	92.6	66.4	92.5	60.7
crown-level	mean (SD)	na	75.8 (9.4)	na	76.1 (15.8)	na	66.3 (5.9)	na	61.6 (1.6)	na	56.7 (18.0)
	best SVM	na	92.5	na	79.2	na	68.4	na	65.2	na	59.0

Table 4.1. Liana cover classes, training data proportion, and number of crowns for each binary threshold classification. The SVM 5-fold cross validation and balanced accuracy are presented for the pixel-level and the crown-level (where applicable).
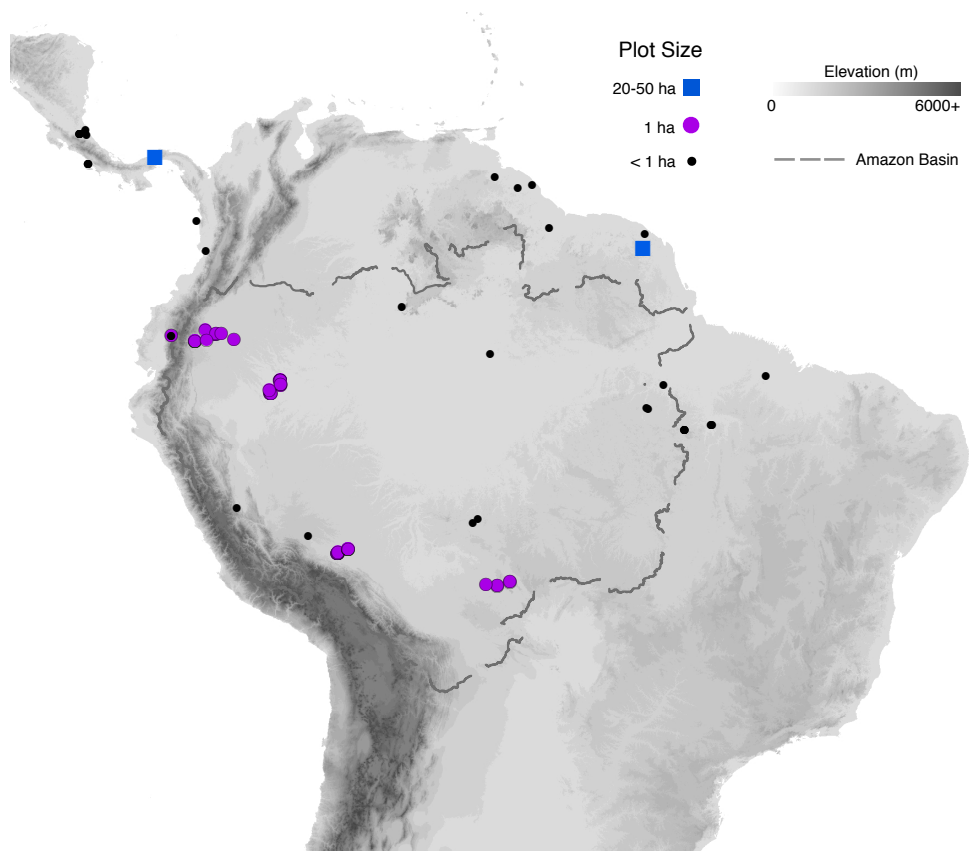
Figure 4.1. Geographic location of plots (Source: citations in text) where lianas have been censused at two different time periods in Central and South America (note: some locations contain multiple plots). Symbols indicate plot location and size of plot. Dashed line denotes the extent of the Amazon basin (Source: www.ore-hybam.org). Elevation data (1km) from NASA Shuttle Radar Topography Mission.
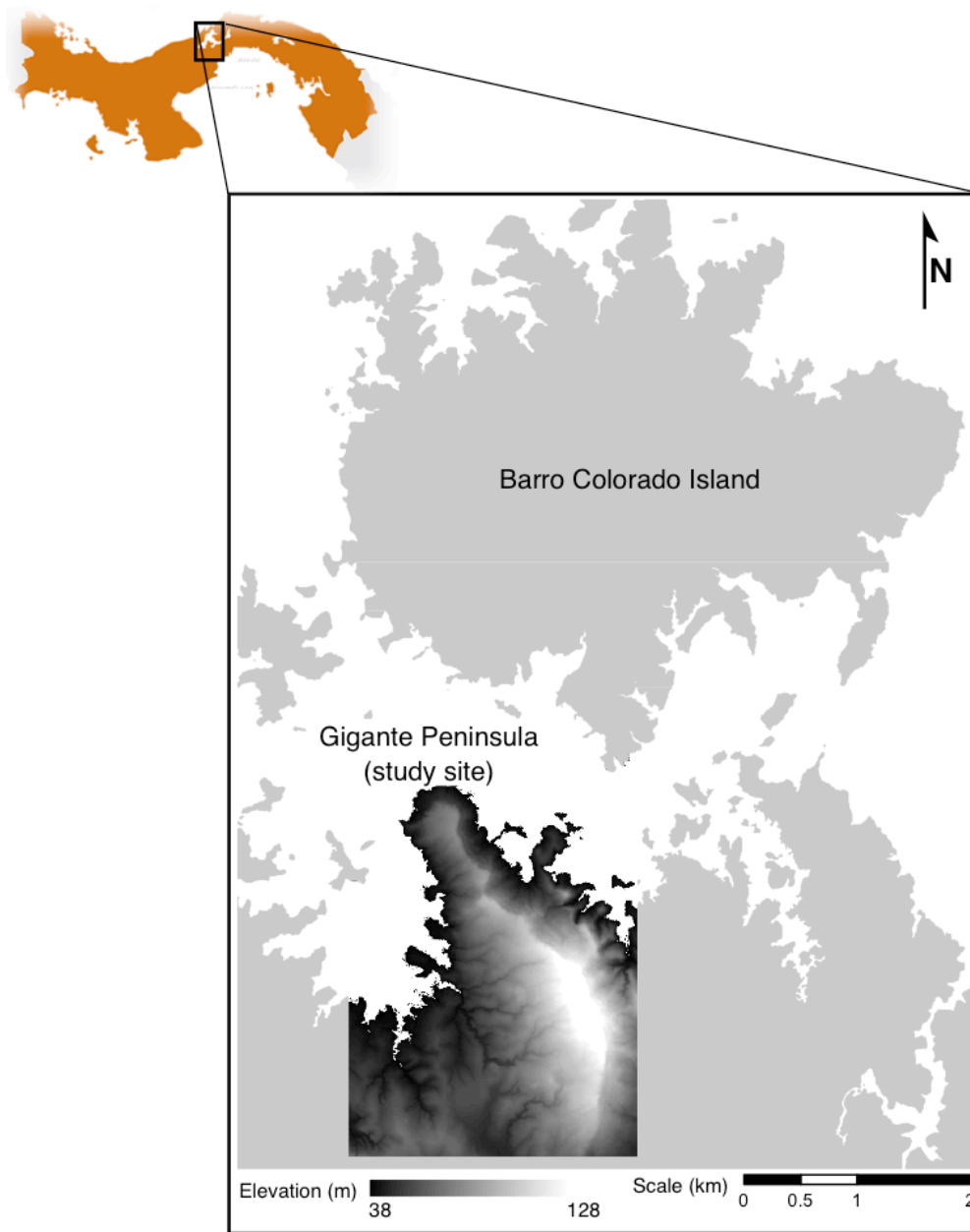
Figure 4.2. Gigante Peninsula study site in central Panama. A LiDAR-derived digital elevation model (DEM) is displayed over the extent of the study site.
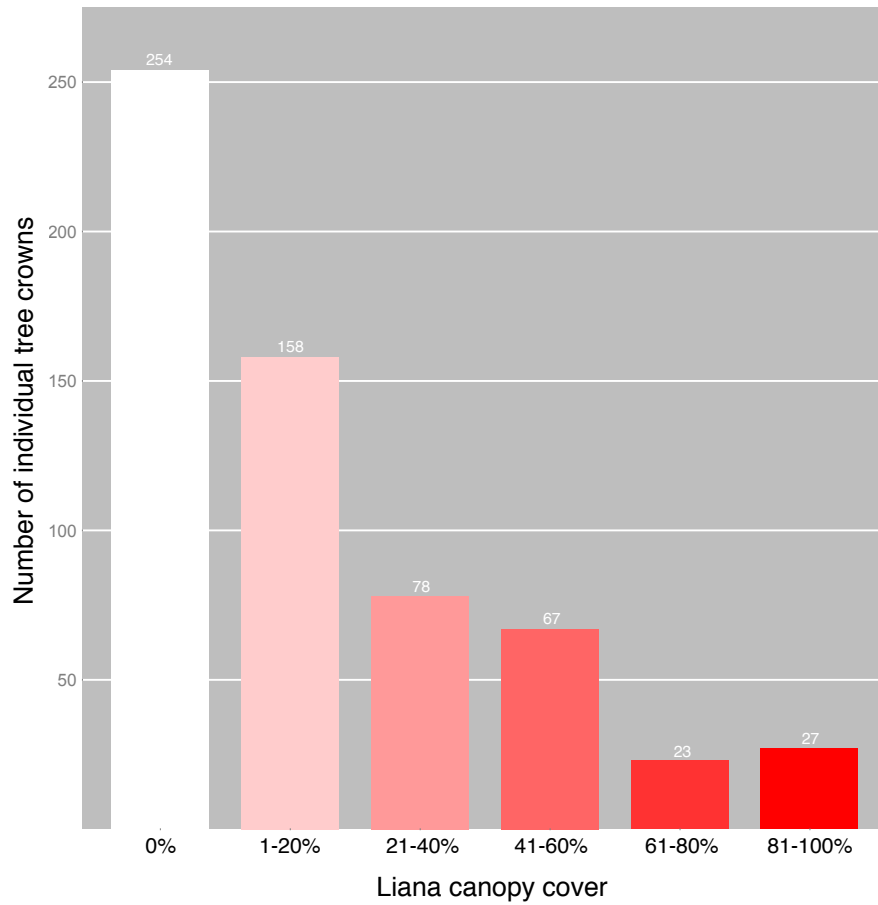
Figure 4.3. Distribution of individual tree crowns at the central Panama study site by level of liana canopy cover as determined by our field survey.
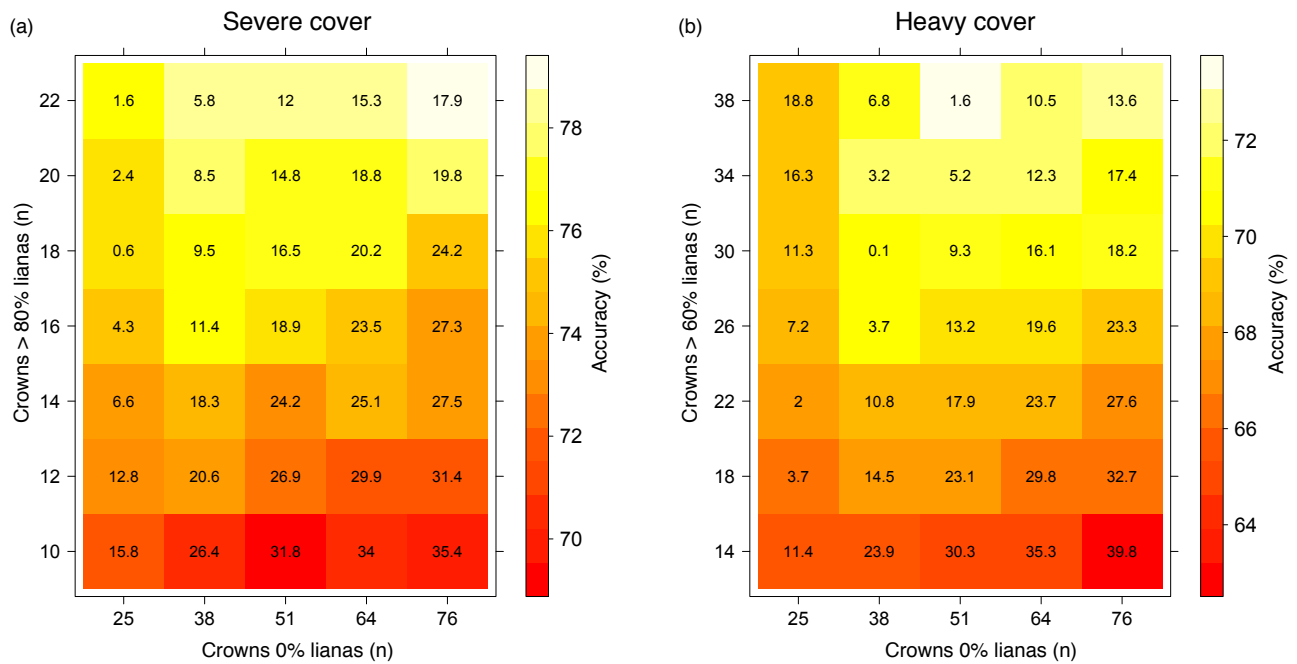
Figure 4.4. Balanced accuracy plotted by color (with standard deviation overlaid) resulting from 100 SVM model iterations of randomly selected tree crowns in the (a) the severe cover and (b) heavy cover binary threshold classifications. Data not shown for the moderate, mild, and thin binary threshold classifications.
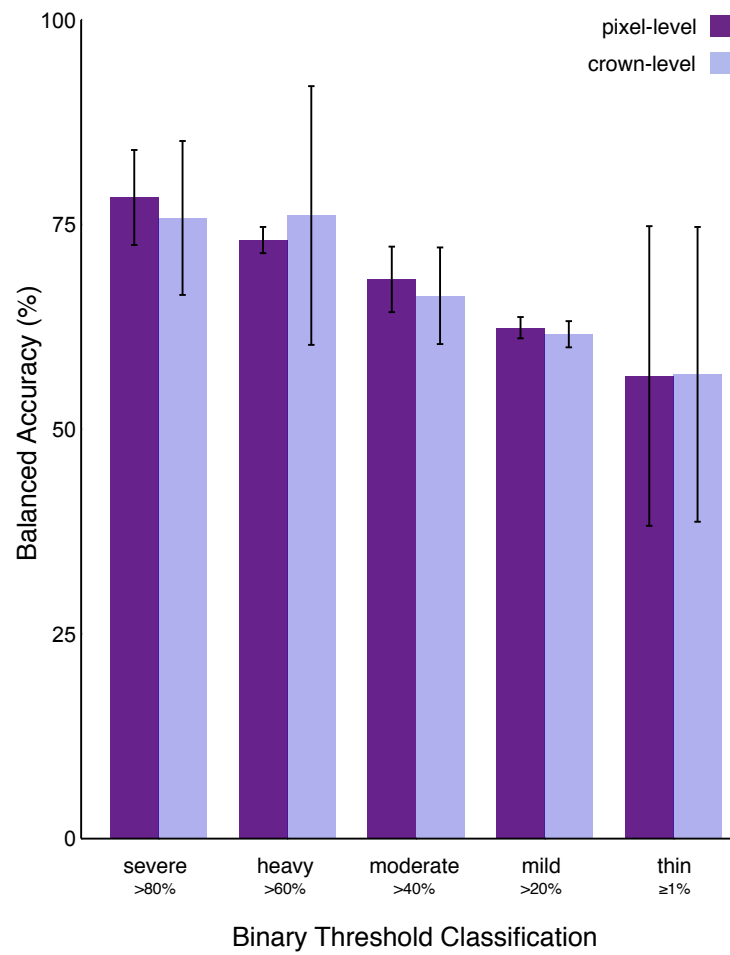
Figure 4.5. Comparison of BAC among binary threshold classifications. Pixel-level accuracy (dark purple) and crown-level accuracy (light purple) with error bars +/-SD.
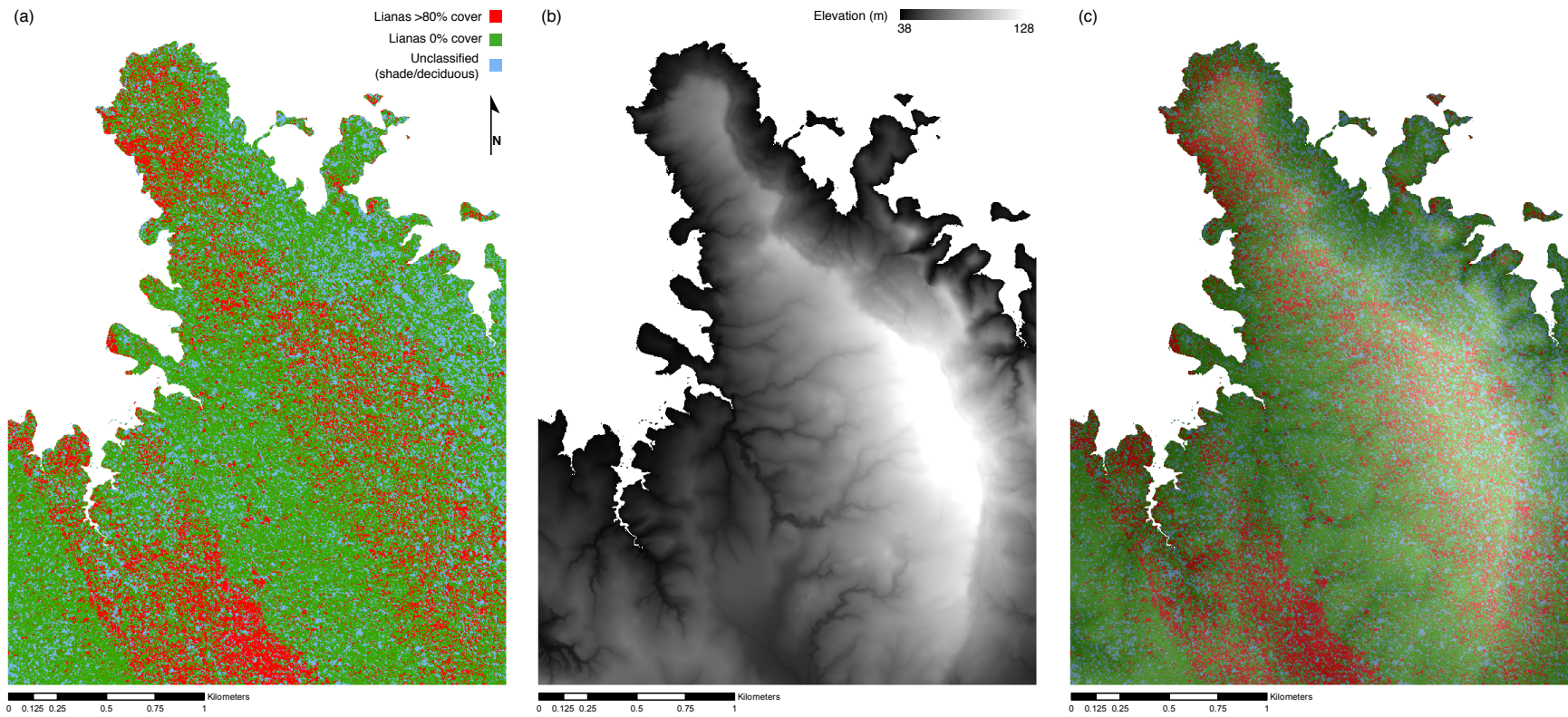
Figure 4.6. (a) Landscape liana severe cover classification of the full VSWIR image resulting from the optimal full SVM, (b) LiDAR-derived digital elevation model (DEM), (c) transparent classification layer overlaid on top of the DEM.

Spectral Region	Mechanisms Controlling Reflectance	Relevance to Liana-Tree Discrimination		
		Leaf-level	Canopy-level	Physiology
Visible	Chlorophyll and carotenoid concentrations[1]	Higher reflectance of lianas in green peak important[3,4,5]	Higher reflectance of lianas found with increasing abundance[2,5]	Lianas contain lower pigment levels[2,3,4]
Red Edge	Chlorophyll content, biomass, water stress[1]	Higher reflectance of lianas in red edge important[3,4,5]	Same as visible but less important for discrimination[2,5]	Lianas contain lower chlorophyll[2,3,4]
Near Infrared	Leaf internal structure, nitrogen concentration[1]	Results are mixed; spectral and physiological differences are inconclusive[2,3,5,6]		
Shortwave Infrared	Leaf water content, cellulose[1]	Most important region for discrimination[5,6]	By far the most important region for discrimination[5]	Leaf water content higher in lianas[4,6]

Table S4.1. A review of studies examining liana and tree spectral differences in relation to leaf physiology.

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Chapter V

Conclusion and Synthesis

While lianas have long fascinated biologists (Darwin, 1865), only recently have we begun to reveal the full extent of their ecological importance. As the second most dominant woody plant growth form in tropical forest ecosystems, lianas are integral to many aspects of forest ecosystem function. Lianas provide abundant food resources and shelter for animals, and ground-to-canopy and canopy-to-canopy pathways for arboreal animals (Emmons & Gentry, 1983; Clay *et al.*, 2010). On the other hand, by increasing tree mortality (Phillips *et al.*, 2002; Ingwell *et al.*, 2010), reducing tree growth and fecundity (e.g., Nabe-Nielsen *et al.*, 2009; van der Heijden & Phillips, 2009), and suppressing regeneration of trees in forest gaps (Toledo-Aceves & Swaine, 2008; Schnitzer & Carson, 2010), lianas are important to whole-ecosystem carbon flux. It is precisely these negative effects that make the reported relative increase of lianas compared to trees such a concern for the world's greatest terrestrial carbon sink: neotropical forests. This dissertation has focused on understanding the mechanisms responsible for the liana increase and the scale on which it is occurring.

Using a combination of empirical experimentation, ground-based forest censuses, and advanced airborne remote sensing imagery and analysis, this work has contributed two key findings. First, that increasing atmospheric CO_2 and its interaction with either seasonal drought or soil nutrient availability are unlikely the main underlying mechanisms responsible for

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increasing lianas (Chapter 2 and 3). More empirical work, included species-specific tests of

elevated CO_2, is needed to form a mechanistic understanding of the processes driving lianas to

become more abundant, relative to trees. Second, I have developed new methods to detect liana

canopy cover at the landscape scale, which include the tools to verify, quantify, and monitor

liana canopy cover in a spatially explicit manner (Chapter 4). Large-scale, high-resolution maps

of liana canopy cover can be used to examine the temporal and spatial ecology of lianas and,

when combined with field and other remote sensing data, document their effects on ecosystem

function at an unprecedented scale. I review in more detail below the contribution of each

chapter and the future questions each has provoked.

**Chapter 2: No evidence that elevated CO_2 gives tropical lianas an advantage over

tropical trees**. While elevated CO_2 is often cited as a main cause of increasing lianas, and is

grounded in sound physiological and ecological theory, there have been few empirical tests of

the effect of CO_2 on tropical lianas. In the first experiments to directly compare the relative

response of lianas and trees to elevated CO_2, I tested a) the response of 11 tropical liana and 10

tropical tree species to increased atmospheric CO_2 and b) whether seasonal drought affected the

response of each growth form. Both lianas and trees had a significantly positive response to

elevated CO_2, but their relative responses did not differ. If elevated CO_2 was the main

mechanism responsible for increasing lianas *as a growth form* we should expect at least some

difference in their growth and physiological responses even at the seedling stage. I emphasize

lianas as a *growth form* because what is often lost in the conversation about liana-tree dynamics

in the context of increasing lianas is the essential role of species-specific differences. While my

data analyses thus far have not examined the response to elevated CO_2 at the species level in

either growth form, I acknowledge the need for doing so and the important questions we can

answer as a result. Indeed, investigations into the mechanistic understanding of increasing lianas, as well as censuses of temporal demographic changes, must be performed at the species level. These two approaches, experimentation and field censuses, can inform one another, with demographic census data used in the selection of species for experimentation, and experimental results used to design field studies testing these mechanisms in natural systems.

Chapter 3: The relative growth response of tropical lianas to elevated CO_2 does not depend on soil nutrient availability. Ecological processes do not occur in isolation, therefore I extended the tests of liana-tree response to CO_2 to include soil nutrient availability because nutrients are key constraints on plant growth and productivity. In two separate studies, tropical liana seedlings were grown in pots with either low or high soil nitrogen (N) or phosphorus (P), and exposed to elevated and ambient CO_2. Counterpart tree species were also tested in the nitrogen experiment. Our experiments did not provide any evidence that soil N and P availability interacts with increasing atmospheric CO_2 to significantly affect the growth and physiology of the liana species studied. Instead, changes to soil nutrient availability or to CO_2 alone had strong and significant effects on lianas. This suggests that while explanations for the reported liana increase may not lie in the interaction among changing biogeochemical cycles, changes of these cycles alone could be a potential contributor. Further experimentation on a wider range of species of lianas and trees, older individuals, and on competition between the growth forms for soil nutrients are needed to clarify whether changes in nutrient availability could be a cause of increasing lianas. Additional belowground variables such as microbial feedbacks, pathogen effects, and other constituent soil nutrients other than N and P should be examined for their influence on liana and tree growth either alone or in concert with elevated CO_2.

Chapter 4: Mapping liana canopy cover across tropical forest landscapes using high-resolution imaging spectroscopy. Studies documenting the relative increase of tropical lianas over trees have generated much attention, yet there still remains a lack of data to verify the existence, scale, and severity of the increase. The use of remote sensing to discriminate between the cover of lianas versus trees in tropical forests canopies provides a viable solution. I used data collected from my field surveys to train machine learning algorithms to detect liana coverage in tree canopies using high-resolution hyperspectral imagery. This method proved to be very accurate at distinguishing liana cover from tree cover, at least for severe liana infestations. When applied to a 600 ha contiguous tropical forest in central Panama, we demonstrate that trees with liana coverage of >80% constituted 11.9%-18.0% of the landscape. Liana infestation of this magnitude, even over relatively small areas, has broad implication for future forest ecosystem function and carbon dynamics. Our newly developed method needs to be applied and verified across a diversity of tropical forest locations and types before we can begin to characterize the temporal and spatial ecology of lianas in neotropical forests. By combining estimates of liana cover at large scales with data on carbon density, species demography, and edaphic variables at the plot-level, we can reveal the biotic and abiotic factors shaping liana community dynamics and how lianas in turn affect forest function.

The very nature of ecological interactions requires that their study be multi-scale, temporal, and involve diverse investigative approaches. However, not every ecological mechanism operates across all scales of space, time, and phylogeny. I have shown here that even seemingly obvious explanations for a phenomenon cannot be verified once tested. Results of this nature are an important step in identifying a specific mechanism and the scale at which it operates. My dissertation suggests that increasing atmospheric CO_2 is unlikely the mechanism

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behind the reported increase in liana size and abundance. At the same time, I have developed new tools for investigating alternative mechanisms for the liana increase. The study of anthropogenic global change in an ecological context requires the flexibility to develop and employ new such methods as the results dictate.

Elevated CO_2 and climate change are a subset of the dramatic effects humans are having on the tropical biosphere. While deforestation rates have slowed in many parts of the Amazon, this decrease has been more than offset by dramatic increases in other tropical regions (Hansen *et al.*, 2013). Worse still, the area subjected to forest degradation (selective logging, fire, mining, and hunting) is 20-30 times that of deforestation alone (Asner, 2013). However, proposals are being seriously considered that would simultaneously tackle the seemingly intractable issues of forest destruction and climate change. Reduced Emissions from Deforestation and Degradation (REDD+) seeks to incentivize tropical forest conservation by linking carbon emitters to parties able to reduce forest carbon losses (Stickler *et al.*, 2009). The efficacy of REDD+ will depend in part on the ability of tropical ecologists to comprehensively understand and accurately monitor tropical forest dynamics and growth. Thus, ecologists will continue to play an integral role in solving some of my generation's largest issues, and it is incumbent upon our field to recognize and meet the challenges presented.

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