Old-growth Neotropical forests are shifting in species and trait composition

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Abstract. Tropical forests have long been thought to be in stable state, but recent insights indicate that global change is leading to shifts in forest dynamics and species composition. These shifts may be driven by environmental changes such as increased resource availability, increased drought stress, and/or recovery from past disturbances. The relative importance of these drivers can be inferred from analyzing changes in trait values of tree communities. Here, we evaluate a decade of change in species and trait composition across five old-growth Neotropical forests in Bolivia, Brazil, Guyana, and Costa Rica that cover large gradients in rainfall and soil fertility. To identify the drivers of compositional change, we used data from 29 permanent sample plots and measurements of 15 leaf, stem, and whole-plant traits that are important for plant performance and should respond to global change drivers. We found that forests differ strongly in their community-mean trait values, resulting from differences in soil fertility and annual rainfall seasonality. The abundance of deciduous species with high specific leaf area increases from wet to dry forests. The community-mean wood density is high in the driest forests to protect xylem vessels against drought cavitation, and is high in nutrient-poor forests to increase wood longevity and enhance nutrient residence time in the plant. Interestingly, the species composition changed over time in three of the forests, and the community-mean wood density increased and the specific leaf area decreased in all forests, indicating that these forests are changing toward later successional stages dominated by slow-growing, shade-tolerant species. We did not see changes in other traits that could reflect responses to increased drought stress, such as increased drought deciduousness or decreased maximum adult size, or that could reflect increased resource availability (CO_2, rainfall, or nitrogen). Changes in species and trait composition in these forests are therefore most likely caused by recovery from past disturbances. These compositional changes may also lead to shifts in ecosystem processes, such as a lower carbon sequestration and "slower" forest dynamics.

Key words: disturbance; drought; environmental gradients; forest dynamics; functional traits; global change; rainfall; resource availability; soil fertility.

Introduction

Tropical forests are of global importance for maintaining biodiversity, storing and sequestering carbon, and regulating the world's climate (Bonan 2008, Alkama and Cescatti 2016). Evidence continues to grow, however, that these forests are not in stable state (Heckenberger et al. 2003) but are undergoing large-scale changes in species composition and dynamics (Brienen 2015), which may be attributed to various global change drivers (Wright 2005). To predict the future of old-growth forests, a better understanding is needed of the direction of forest change and its underlying drivers. One way to achieve this is by evaluating community-level changes in functional traits. Here, we evaluate changes in species composition and 15 leaf, stem, and whole-plant traits among five Neotropical forests and infer the underlying global drivers by analyzing whether and how traits change.

Spatial variation in species and trait composition

Species distributions are among others determined by species' responses to climate (Engelbrecht et al. 2007) and soil conditions (Clark and Palmer 1999, Toledo et al. 2012). Such species-specific responses in distribution are associated with species' traits which ultimately determine species' strategies to acquire and use resources (Violle et al. 2007). Analysis of shifts in traits in relation to environmental conditions (also referred to as "response traits"; Suding et al. 2008) are therefore expected to provide mechanistic insights into the underlying drivers of change. Many studies have addressed the effect of environmental conditions on species composition and community-level trait values for grasslands (Pakeman 2004) and individual forests (Feeley et al. 2011, Fauset et al. 2012). These studies generally find that the values of community-level traits respond to environmental gradients. However, environmental conditions vary more at larger spatial scales (e.g., across the Neotropics), leading to strong species turnover. As a result, the composition of species, and thus the composition of traits, should differ more strongly at large than at local scales. Few studies have addressed community-level changes across large-scale environmental gradients, and studies that do exist tend to focus only on a few traits (e.g., Baker 2004, Wright 2004). Here, we evaluate changes in 15 traits for five forests spanning large environmental gradients from Bolivia to Costa Rica to test the hypothesis that differences in community-mean trait values among forests are a result of gradients in environmental conditions.

Temporal variation in species and trait composition

Old-growth tropical forests are not in stable state. Natural or anthropogenic disturbances can set back a forest to an earlier successional state, causing community reassembly (Chazdon 2003). Moreover, global change, such as increased atmospheric CO_2 concentrations or increased drought stress can alter species composition, eventually pushing the forest to an alternative stable state. Several studies have demonstrated changes in species composition over the last decades, although results and hypothesized drivers are contradictory, which could be caused by differences among sites in changing environmental conditions. Some studies find an increase in the abundance of drought-tolerant and deciduous species possibly due to increasing (atmospheric) drought stress as caused by decreased rainfall and/or increased temperature (Enquist and Enquist 2011, Feeley et al. 2011, Fauset et al. 2012, Zhou et al. 2014). Other studies find an increase in the abundance of emergent and canopy species due to increased resource availability such as CO_2 (Laurance et al. 2004) or recovery from recent disturbances (Nelson 2005), and again others find an increased abundance of slow-growing species with high wood density, indicating that the forest is recovering from more historical disturbances and/or facing a reduction in resource availability (Chave 2008). We aim to obtain a better understanding of possible underlying causes of compositional change by evaluating temporal changes in the community-weighted mean trait values of functional leaf, stem and whole-plant traits across tropical forests.

Questions and hypotheses

We address two questions. First, how do community-weighted mean trait values differ across five Neotropical forests? We expect that an increase in soil nutrient availability would increase the abundance of species with acquisitive trait values (e.g., high specific leaf area and leaf nutrient concentrations) that acquire more resources and grow faster. Trait responses along the precipitation gradient should be determined by drought adaptations at low rainfall, for example by drought-deciduousness, and by shade adaptations at high rainfall. Drought-deciduous species at low rainfall may compensate for their short leaf life span with more acquisitive trait values that lead to faster growth in the short growing season, whereas evergreen species at high rainfall may have conservative trait values to increase leaf life span. Wood traits will be most conservative (e.g., high wood density) at dry sites or at sites with low nutrient availability to reduce drought cavitation and increase wood resistance to pathogens (Muller-Landau 2004, Romero and Bolker 2008, Markesteijn et al. 2011b).

For the second question we ask how species composition and community-weighted mean trait values change over time. We identify three important environmental change drivers that should favor species with certain trait values more than others, leading to changes in the community-mean trait values (Tables 1 and 2):

1) Increased resource availability (e.g., CO_2 and nutrient deposition; Laurance et al. 2004, Hietz et al. 2011) would increase the abundance of i) species with acquisitive trait values that can make use of the increased availability of resources, ii) species with a tall adult stature that are better competitors for aboveground resources (i.e., light) in a denser forest canopy, and, iii) in the case of nutrient deposition, reduce the Fabaceae abundance because of reduced advantage from N_2-fixation. Such changes in community-weighted mean trait values could also be observed in response to recent disturbances, such as wind storms, which open up the canopy and favor the establishment of acquisitive species.

2) Increased drought stress (through decreased rainfall and/or increased temperature) would increase the abundance of i) drought-avoiding, deciduous species that generally have high specific leaf area (Enquist and Enquist 2011), ii) physiologically drought-tolerant species with high wood density that are cavitation resistant (Markesteijn et al. 2011b), iii) species with a small adult stature that suffer less from water transport limitations (Bennett

TABLE 1. Trait abbreviations, descriptions, units, and an explanation of what the trait indicates.

Abbreviation	Description	Units	Indicator of
SLA	specific leaf area	cm^2/g	light interception efficiency
LA	ln-transformed leaf area	cm^2	light interception, heat balance
N$_{leaf}$	leaf nitrogen concentration	%	photosynthetic capacity
P$_{leaf}$	leaf phosphorus concentration	%	growth and photosynthetic capacity
N:P$_{leaf}$	leaf nitrogen:phosphorus ratio		relative nutrient limitation
Chl	leaf chlorophyll content	μg/cm^2	light-harvesting capacity
LDMC	leaf dry matter content	g/g	leaf defense
FP$_s$	specific force to punch	N/cm^2	leaf defense
LMF$_m$	leaf mass fraction of the metamer	g/g	light interception efficiency
WD	wood density	g/cm^3	stem defense, drought tolerance
DBH$_{max}$	95% quantile of stem diameter for all individuals per species	cm	tree longevity and life history strategy
CE$_{max}$	95% quantile of crown exposure index for all individuals per species	index (1–5)	tree longevity and life history strategy
% Fab	percentage of individuals from Fabaceae	%	N-fixing capacity
% Compound	percentage of individuals with compound leaves	%	heat balance
% Deciduous	percentage of individuals that are deciduous	%	drought avoidance

et al. 2015), and iv) species with small leaves that allow for a better convective heat cooling.

3) Recovery from past disturbances should cause a shift from early-successional species with acquisitive trait values toward late-successional species with more "conservative" trait values and tall adult stature, whereas Fabaceae should become less abundant due to decreased N limitation in older forests (Batterman et al. 2013, Sullivan et al. 2014).

METHODS

Sites

We used data from permanent sample plots in five Neotropical forests, spanning a large latitudinal gradient in the Neotropics (from 16°07′ S in Bolivia to 10°12′ N in Costa Rica, see the map in Appendix S1), and broad gradients in rainfall (1160–3900 mm/yr) and soil conditions (Table 3). From low to high annual precipitation, we used two forest sites in Bolivia (INPA and La Chonta), one in Brazil (Tapajós), one in Guyana (Pibiri), and one in Costa Rica (Corinto). These forests also differ in soil fertility, from young and fertile soils in La Chonta to old and poor soils in Pibiri. Hereafter, these forest sites will be referred to as dry deciduous (DD; INPA), moist semideciduous (MSD; La Chonta), moist evergreen (ME; Tapajós and Pibiri), and wet evergreen (WE; Corinto).

Plot design

We used permanent plots in old-growth forests that were not disturbed by human activities or fire during the time of monitoring. To facilitate comparisons across sites, we used a similar time window for all sites (2000–2013), a plot size of 1 ha (if available), and included all trees ≥10 cm DBH.

The plots in the dry deciduous site (INPA) were established and all trees ≥10 cm DBH were identified and measured by Instituto Boliviano de Investigación Forestal (IBIF). The plots in the moist semideciduous site (La Chonta) were also established and measured by IBIF. The plots in the moist evergreen forest of Tapajós were established and all trees ≥5 cm DBH were identified and measured by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). To use the same diameter limit as for the other sites, we used only trees ≥10 cm DBH. Besides the time window of about 10 yr, we included an analysis of longer-term changes (29 yr) for Tapajós. The plots in the moist evergreen forest of Pibiri were established and measured by Tropenbos. All trees ≥20 cm DBH were measured in the whole plot and trees ≥5 cm were measured in 25 subplots that in total covered an area of 0.25 ha/plot. We considered the trees between 10 and 20 cm DBH, which were measured on 0.25 ha/1-ha plot, four times (to scale to 1 ha). The plots in the evergreen wet forest (Corinto) were established and all trees >10 cm in DBH were measured by Centro Agronómico Tropical de Investigación y Enseñanza (CATIE).

Trait collection

Here we provide a short description of the collection of traits (see Table 3 for references providing more detailed information). All traits were expressed at the plot level and, in general, traits were measured according to standard protocols (Pérez-Harguindeguy 2013). We measured traits that are important for the carbon, water, nutrient, and heat balance of the plant (Table 1), and hence should respond to global change drivers. We used specific leaf area (SLA), leaf area (LA), leaf nitrogen (N$_{leaf}$) and phosphorus concentration (P$_{leaf}$), leaf N:P ratio (N:P$_{leaf}$), leaf chlorophyll content (Chl), leaf dry matter content (LDMC), specific force to punch (FP$_s$),

TABLE 2. Hypothesized temporal changes in 15 community-weighted mean (CWM) traits in response to three potential drivers of environmental change: increased resource availability, increased drought stress, and recovery from past disturbances. The last row shows the observed changes in CWM traits across the five forest sites.

Driver	SLA	LA	N_{leaf}	P_{leaf}	$N:P_{leaf}$	Chl	LDMC	FP_s	LMF_m	WD	DBH_{max}	CE_{max}	%Fab	% Compound	% Deciduous
Increased resource availability	↑	–	↑	↑	↑	↑	↓	↓	↓	↓	↓	↑	↓	↓	↓
Increased drought stress	↑	↓	–	–	–	–	↑	–	↓	↑	↓	↓	↑	↑	↑
Recovery from past disturbance	↓	↓	↓	↓	↑	↓	↑	↑	↓	↑	↑	↑	↓	↓	–
Observed changes	↓	(↓)†	–	–	(↑)‡	–	–	–	(↑)§	↑	(↑)¶	(↑↓)#	(↓)‖	–	–

Notes: The traits used are specific leaf are (SLA), leaf area (LA), leaf nitrogen concentration (N_{leaf}), leaf phosphorus concentration (P_{leaf}), leaf N:P ratio ($N:P_{leaf}$), leaf chlorophyll content (Chl), leaf dry matter content (LDMC), specific force to punch (FP_s, a measure for leaf toughness), leaf mass fraction (LMF_m), wood density (WD), species-specific maximum diameter (DBH_{max}), species-specific maximum crown exposure index (CE_{max}), percentage of individuals belonging to Fabaceae (% Fab), % of individuals with compound leaves (% Compound), and percentage of individuals that are deciduous (% Deciduous). See Table 1 for more details about trait measurements. In the first three rows, hypothesized positive changes are shown by ↑, hypothesized negative changes by ↓, and no hypothesized changes by a "–." In the last row, ↑ indicates a consistent increase over sites, ↓ a consistent decrease over sites, arrows in between brackets an increase or decrease for part of the sites, and – no significant temporal changes for any of the sites.

† Decrease for the moist evergreen forest (Tapajós) over 30 yr.
‡ Increase for the dry deciduous forest.
§ Increase for the moist semideciduous forest.
¶ Increase for the moist evergreen forest (Pibiri).
# Increase for the moist semideciduous forest and decrease for the moist evergreen forest (Pibiri).
‖ Decrease for the moist evergreen forest (Tapajós).

TABLE 3. Details of the five forest sites used in this study: INPA, La Chonta, Tapajós, Pibiri, and Corinto.

Characteristic	INPA	La Chonta	Tapajós	Pibiri	Corinto
Coordinates	16°07′ S, 61°43′ W	15°47′ S, 62°55′ W	3°19′ S, 54°57′ W	5°13′ N, 58°38′ W	10°12′ N, 83°52′ W
Country	Bolivia	Bolivia	Brazil	Guyana	Costa Rica
Forest type	dry deciduous (DD)	moist semideciduous (MSD)	moist evergreen (MEtap)	moist evergreen (MEpib)	wet evergreen (WE)
Number of control plots	8	9	6	3	3
Size of plots (ha)	1 (100 × 100 m)	1 (100 × 100 m)	0.25 (50 × 50 m)	1 (100 × 100 m)	1 (100 × 100 m)
First census	2002–2003	2000–2001	2003 (and 1983)	2000	2000
Last census	2012–2013	2009–2011	2012	2013	2010
Time span (yr)	10	8	9 (and 29)	13	10
Rainfall (mm/yr)	1160	1580	2110	2772	3900
No. dry months < 100 mm rainfall	7	6	3	0	0
Average annual temperature (°C)	24.3	24.3	25	25.9	23.7
Temporal change in annual rainfall (1900–2013)	n.s.	n.s.	n.s.	↑	↑
Temporal change in SPEI (1900–2013)	↓	n.s.	n.s.	↑	↑
Soil type	Oxisols	Ultisols	Oxisols	Ferralsols	Inceptisols
Soil fertility from highest (1) to lowest (5), based on Fig. 2 of Quesada (2010)	2 (middle-high)	1 (high)	3 (middle-low)	4 (low)	n.a.
Sources	Peña-Claros (2012)	Peña-Claros (2012)	de Carvalho (1992), Silva et al. (1995), Aragão (2009)	van Kekem et al. (1996), van der Hout (1999), van Dam (2001)	Sesnie et al. (2009), Finegan (2015)
No. species with leaf and stem trait data	98	158	68	33	72
Plot abundance covered with traits (%), averaged per site	96.7	82.9	72.5	78.6	85.1
Reference for more details on trait collection	Markesteijn et al. (2011)a	van der Sande et al. (unpublished manuscript), Rozendaal et al. (2006), Poorter (2008), Carreño-Rocabado et al. (2012), van Gils (2012)	de Avila et al. (unpublished manuscript)	van der Sande et al. (unpublished manuscript)	

Notes: SPEI, Standardized Precipitation and Evapotranspiration Index, with high values indicating wet conditions; n.a., not available; n.s., not significant; ↑ a significant temporal increase; ↓ a significant temporal decrease.

leaf mass fraction of the metamer (LMF_m), wood density (WD), maximum stem diameter (DBH_{max}), maximum crown exposure index (CE_{max}), percentage of individuals from Fabaceae, percentage of individuals with compound leaves, and percentage of individuals from deciduous species (Table 1).

For each site, we measured leaf and stem traits for the most abundant tree species (on average representing 84% of all individuals in the plots). All leaf traits were measured on about 5 (range 1–10) individuals per species and 4–5 leaves per individual. To have comparable measurements among species and sites, individuals were selected that were growing in relatively open conditions and that had a DBH of 8–20 cm. Trees in this size class are well established and their leaves are still accessible with a pruner on an extension pole. Leaves harvested were healthy and exposed to high-light conditions. LA was measured on fresh leaves without the petiole, and ln-transformed for a normal distribution. SLA was calculated as the fresh leaf area divided by the dry mass (cm^2/g), and was based on the whole leaf (including rachis for compound leaves). Chlorophyll content was defined as mass per unit leaf area ($\mu g/cm^2$) using a SPAD meter (Minolta SPAD 502 Chlorophyll Meter, Spectrum Technologies Inc., Plainfield, IL, USA), N_{leaf} and P_{leaf} (%) as concentrations of dry mass, and $N_{leaf}:P_{leaf}$ provided the $N:P_{leaf}$ ratio. LDMC was calculated by dividing the leaf dry mass by the leaf fresh mass (g/g). FP_s was measured using a penetrometer, which measures the force needed to punch the flat-ended side of a nail through the leaf. FP_s was then calculated by dividing the force needed to punch the leaf by the product of the circumference of the nail and the thickness of the leaf (N/cm^2), to correct for the fracture area on which pressure is exerted. LMF_m was calculated by dividing the leaf dry mass by the sum of the biomass of the whole metamer, i.e., the dry masses of the leaf, petiole and internode (g/g).

To take into consideration the possible radial variation in wood density (Hietz et al. 2013), WD (g/cm^3) was based on the average of the whole stem radius of a tree. Per species, a wood core was taken from about three individuals of 20–40 cm DBH. WD was calculated by dividing the oven-dried mass (for 48 h at 70°C) by the fresh volume. For most species of the moist semideciduous site (La Chonta), wood was collected from the outer sapwood of the tree. These WD values were converted to WD values for the whole radius, based on the relation between WD of the youngest sapwood and WD of the whole radius for 32 Bolivian species ($WD_{radius} = 0.0037 + 1.0607 \times WD_{outer}$; $R^2 = 0.90$; see van der Sande et al. [*unpublished manuscript*]). For Corinto, WD was only measured on the outer sapwood of the tree. DBH_{max} (cm) per species was based on the 95% quantile of diameters for all individuals in a site that were larger than $0.1 \times$ maximum diameter found for that species (King et al. 2006), and CE_{max} was calculated for each species as the 95% quantile of crown exposure values (between 1 and 5; Dawkins and Field 1978) for all individuals in

a site. See Appendix S2 for alternative ways to calculate DBH_{max}. The moist evergreen site (Tapajós) was excluded for CE_{max} because it could not be calculated in the same way.

Community-weighted mean trait composition

To evaluate differences in community-level traits among sites and between census years, we calculated the abundance-weighted mean trait values, also known as the community-weighted mean (CWM; Pla et al. 2012), for the 15 leaf, stem, and whole-plant traits. We weighted by species abundance rather than by species basal area to give equal weight to recruiting and dying trees and, in this way, increase the effect of small, newly recruited trees on changes in mean trait values. A test with basal-area-weighted mean trait values showed similar trends in community-weighted mean trait values (Appendix S3). Hereafter, we therefore only report analyses based on abundance-weighted trait values. Per plot, CWM trait values were calculated based on all live individuals (for which trait data were available) in the first census and all live individuals in the final census. Hence, these resulted in 29 plots × 2 censuses = 58 CWM values per trait. Additionally, we calculated CWM trait values for Tapajós in an earlier census (1983), to evaluate longer-term changes (29 yr). Note that we used mean trait values per species. Therefore, we only evaluate changes in CWM trait values due to changes in species composition, not due to plastic changes in species' trait values over time. Although many species show plastic phenotypic responses within and across individuals to environmental conditions (Poorter et al. 2010), in general the variation explained by intraspecific trait differences is small (12%) compared to interspecific differences (72%, Rozendaal et al. 2006). Sites differed in the number of species with trait data (Table 3). Leaf and stem traits were available for species representing 73–97% (average 84%) of all individual trees per plot.

We also calculated the percentage of individuals of Fabaceae per plot as an indicator of the nitrogen fixing potential (as different subfamilies of Fabaceae have 62% (*Papilionoideae*), 54% (*Mimosoideae*) and 5% (*Caesalpinoideae*) of N_2-fixing genera; Hedin et al. 2009). Furthermore, for each plot and census we calculated the percentage of individuals with compound leaves, and the percentage of individuals that belonged to deciduous species. A species was categorized as deciduous when some (or all) of its individuals possess a yearly leafless period. DBH_{max}, CE_{max}, and Fabaceae abundance were obtained for all species in the plots.

Environmental drivers

Globally, the concentration of atmospheric CO_2 has increased from about 320 ppm in 1960 to almost 400 ppm in 2013 (Appendix S4). Annual rainfall between 1900 and 2013 significantly increased for the

two wettest sites (Corinto and Pibiri) and did not change for the three driest sites (INPA, La Chonta and Tapajós) (Table 3; Appendix S5). The Standardized Precipitation and Evapotranspiration Index (SPEI) is a measure for dryness, with positive values indicating humid conditions and negative values indicating dry conditions. Over the period 1900–2013, SPEI significantly decreased for the dry deciduous site (i.e., it became drier), significantly increased for the two wettest sites (i.e., it became more humid), and did not significantly change for the two intermediate sites (Table 3; Appendix S6). Also over the period 1991–2013 (i.e., the time period in which the data were collected), SPEI values significantly decreased for the dry deciduous site ($P < 0.001$), significantly increased for the wet evergreen site ($P < 0.001$, both only for the 12-month timescale, see Appendix S6), and did not change for the intermediate sites. Hence, the sites do not show consistent increases or decreases in drought. However, extreme drought events (the lowest peaks in Appendix S6) occur repeatedly (with a monthly SPEI value <−2 occurring every 3–8 yr, based on a 12-month timescale), and may therefore still cause changes in species and trait composition.

Statistical analyses

To evaluate how trait composition (i.e., the multivariate CWM trait space) and single CWM trait values differ among sites and change over time (between the censuses) we performed several analyses. Differences in the multivariate CWM trait composition among the five sites and between the first and final census were tested using a redundancy analysis, using the 10 traits that were collected at all sites (see Fig. 1B). Site and census were included as constrained axes, to test for differences in multivariate CWM trait composition. The significance of the constrained axes was tested using a permuted ANOVA, by allowing permutations within plots (Oksanen 2011). To evaluate whether annual precipitation and soil fertility could explain differences between sites (because we do not have variation among plots and between census years), we repeated the analysis twice: one time to include annual precipitation and one time to include soil fertility as the constrained axis instead of site. Soil fertility was based on the ranking from low to high soil fertility between sites (Table 3). The wet evergreen forest was given the highest soil fertility because this forest is growing on volcanic soils (Finegan 2015). Both soil fertility and annual precipitation were included as continuous variables.

For each univariate trait, differences in CWM values among the five sites and between censuses were evaluated using a linear mixed model, with site, census and their interaction as fixed factors, and plot as random factor (to account for census as repeated measures per plot). In case of significant effects of site and/or the interaction of site and census, Tukey's post hoc test was used for multiple comparisons. To test whether the observed changes over a decade were also found for a longer time period, we evaluated temporal changes in CWM trait values in Tapajós between 1983 and 2012, using a linear mixed model with census as explanatory variable and plot as random factor. To evaluate associations among CWM trait values, we used a principal component analysis on
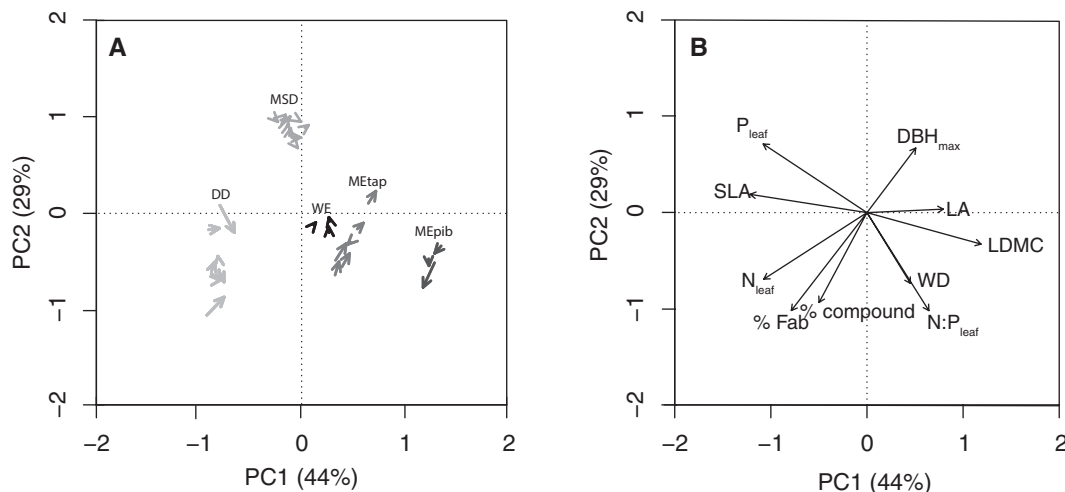

Fig. 1. Multivariate trait composition for plots in two census years in the five sites, based on (A) 10 community-weighted mean traits and (B) associations among community-weighted mean traits. The arrows in panel (A) show the change in multivariate trait composition over the ~10 yr/plot. The different shades of gray indicate the sites, ordered from dry deciduous (lightest gray) to wet evergreen (black): dry deciduous (DD, INPA), moist semideciduous (MSD, La Chonta), moist evergreen (MEtap, Tapajós and MEpib, Pibiri), and wet evergreen (WE, Corinto; Table 3). For trait abbreviations in panel (B), see Table 1. Sites differed significantly in multivariate trait composition ($F_{4,51} = 78.1$, $P = 0.018$), but census did not ($F_{1,51} = 0.3$, $P = 0.609$). Chl, FP_s, LMF_m, CE_{max}, and % deciduous were left out of these analyses because of missing values for some sites. Percentages behind the axes are the variation explained by the principal component axes.

TABLE 4. *P* values from ANOVAs for each community-weighted mean (CWM) trait, with census year (first census around 2000 vs. last census around 2010, Table 3), site, and the interaction between census and site as explanatory variables.

CWM trait	Site	Census	Census × Site
SLA	**<0.001**	**<0.001**	0.052
LA	**<0.001**	0.560	0.151
N_{leaf}	**<0.001**	0.597	0.567
Pl_{leaf}	**<0.001**	0.129	0.325
$N:P_{leaf}$	**<0.001**	0.056	**0.004**
Chl	**<0.001**	0.152	0.345
LDMC	**<0.001**	0.493	0.408
FP_s	**<0.001**	0.086	0.065
LMF_m	**<0.001**	**0.004**	**0.001**
WD	**<0.001**	**0.001**	0.214
DBH_{max}	**0.040**	0.143	**<0.001**
CE_{max}	0.232	0.885	**<0.001**
% Fab	**<0.001**	0.947	**<0.001**
% Compound	**<0.001**	0.928	0.227
% Deciduous	**<0.001**	0.080	0.082

Notes: See Appendix S8 for multiple comparisons among sites for the CWM traits that had no significant interaction between census and site, and see Appendix S9 for multiple comparisons for the CWM traits that had a significant interaction between census and site. LA was ln-transformed. Significant P values are shown in bold.

the centered (with a mean of 0) and standardized (by dividing the centered trait values by their standard deviations) trait values.

To evaluate whether the first and last census differed in species composition, we applied a redundancy analysis to the species abundance data, with census as the constrained axis and permutations within plots. This analysis was done for each site separately, since species composition was too different to be able to combine sites. For Tapajós, a change in species composition was also tested between the years 1983 and 2012.

All analyses were performed in R version 3.1.2. Linear mixed models were performed with the *lme* function of the *nmle* package (Pinheiro and Bates 2016), and multiple comparisons with the *glht* function of the *multcomp* package (Hothorn et al. 2014). Redundancy and principal component analyses were performed with the *rda* function, and the ANOVA to test for constrained axes with the *anova.cca* function, both of the *vegan* package (Oksanen et al. 2014).

Results

The multivariate composition of 10 CWM traits (that were collected at all sites) differed significantly among sites ($F_{4,51}$ = 78.1, *P* = 0.018; Fig. 1A), and also with annual precipitation ($F_{1,54}$ = 20.7, *P* = 0.012; not shown in Fig. 1) and soil fertility ($F_{1,54}$ = 16.3, *P* = 0.012). All individual CWM traits differed significantly among sites, except for CE_{max} (Table 4; Appendix S7). In general, SLA, N_{leaf}, P_{leaf}, and percentage of deciduous species

increased toward drier forests (except for the wettest forest where SLA and leaf nutrients were high, Fig. 2; Appendix S8). In contrast, LDMC, FP_s, and leaf area increased toward wetter forests. In combination, this indicates that leaf trait values tend to be more acquisitive in dry forests. Chl, $N:P_{leaf}$, and LMF_m showed an optimum with rainfall, whereas the other traits did not show a clear pattern with rainfall.

Species composition of the three driest forests (INPA, La Chonta, and Tapajós) changed significantly over time (Table 5, Fig. 3). The shift in species composition toward the center of Fig. 3 indicates a directional convergence of plots over time. Multivariate trait composition did not change significantly over time ($F_{1,51}$ = 0.35, *P* = 0.609; Fig. 1A), but individual traits did (Table 4). Across all sites, specific leaf area decreased and wood density increased over time (Fig. 2). Five traits (DBH_{max}, CE_{max}, $N:P_{leaf}$, LMF_m, and percentage of individuals in Fabaceae) changed over time for one or two sites only (Fig. 2; Appendix S9).

For the moist evergreen forest of Tapajós, we could evaluate longer-term (29 yr) changes. We found significant changes over time in species composition (Table 5), marginal changes in multivariate trait composition (*P* = 0.093, $F_{1,9}$ = 0.665), a significant increase in DBH_{max} and WD, and a decrease in LA and the percentage individuals in Fabaceae and with compound leaves (Appendix S10). Hence, in both the short term (10 yr) and longer term (29 yr), WD and DBH_{max} increased, and Fabaceae abundance decreased for this forest.

Discussion

We evaluated how old-growth tropical forests vary in their community-weighted mean (CWM) trait composition, and whether their species and trait composition changed over time. Multivariate CWM trait composition and individual CWM traits differed strongly among the five Neotropical sites. Species composition changed over

TABLE 5. The effect of census year (as the constrained axis of the redundancy analysis) on species composition, tested using a permuted ANOVA per site (La Chonta, INPA, Tapajós, Pibiri, and Corinto) (Oksanen 2011).

Site	Var	*F*	*P*
Dry deciduous (INPA)	59.40	0.22 (1, 13)	**0.030**
Moist semideciduous (La Chonta)	26.27	0.22 (1, 15)	**0.006**
Moist evergreen (Tapajós 10 yr)	6.46	0.35 (1, 9)	**0.016**
Moist evergreen (Pibiri)	34.70	0.03 (1, 3)	1.000
Wet evergreen (Corinto)	41.50	0.08 (1, 3)	0.625
Moist evergreen (Tapajós 29 yr)	14.14	0.70 (1, 9)	**0.016**

Notes: For each site, the variance (Var), *F* value, and *P* value are given. "Tapajós 29 yr" compares the species composition over a 29-yr time interval. Significant P values are shown in bold.
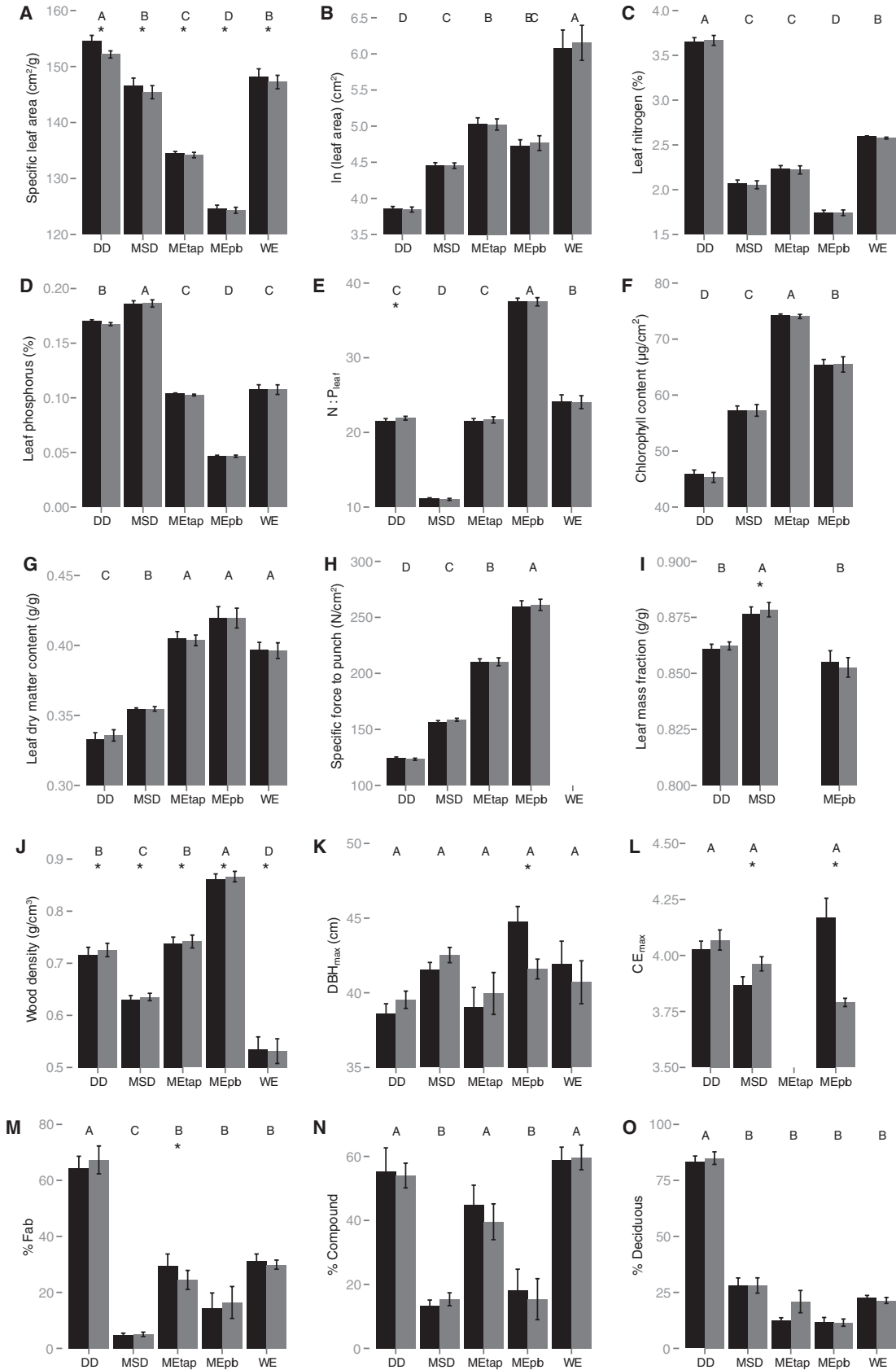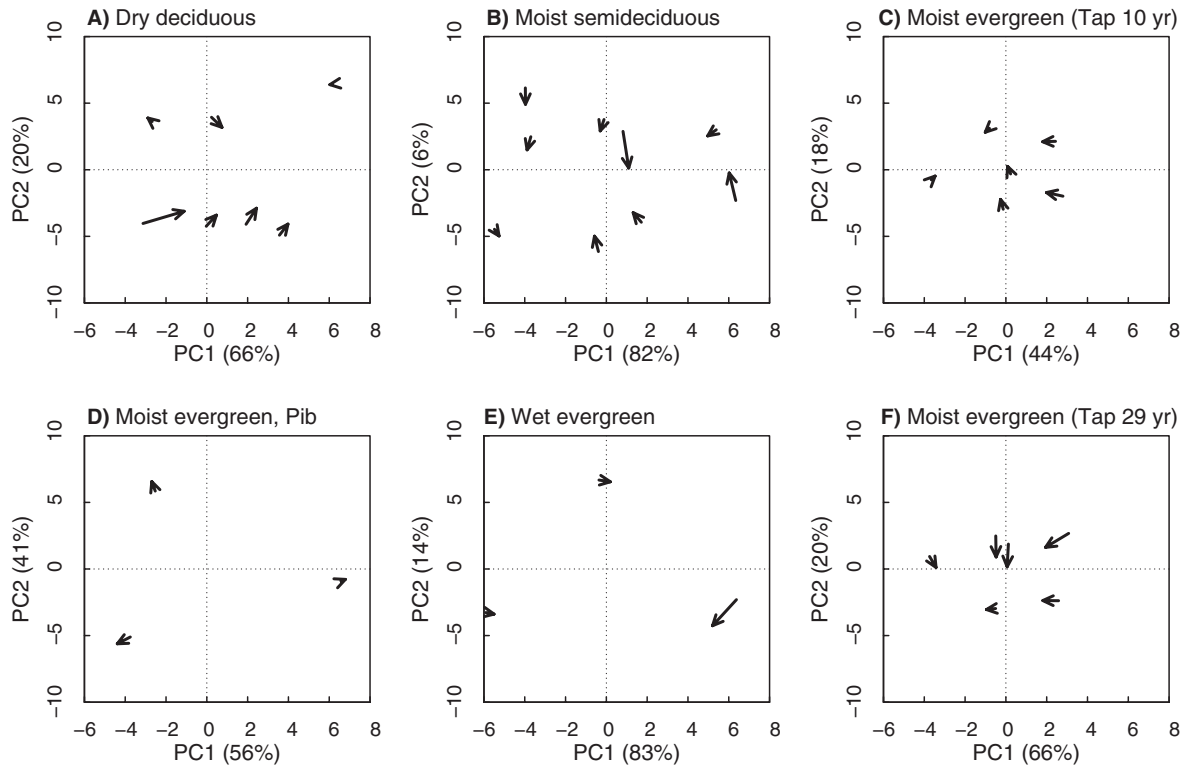
FIG. 3. The temporal change in species composition over ~10 yr for the five sites: (A) dry deciduous, (B) moist semideciduous, (C) and (D) moist evergreen (Tapajós and Pibiri), (E) wet evergreen (Table 3), and (F) over 29 yr for the moist evergreen forest (Tapajós). The arrows show the unconstrained positioning of plots in the first census (the start of the arrow) and last census (the tip of the arrow) along the first and second principal component axes. Percentages behind the axes are the variation explained by the principal component axes. See Table 5 for statistics on temporal changes in species composition.

time for the three driest sites, and several CWM key traits changed significantly over time for all sites.

Strong differences in trait composition among Neotropical forests

The five sites differ strongly in rainfall and soil fertility (Table 3; Quesada 2010) and we therefore expected that they would differ strongly in their multivariate trait composition (i.e., the multivariate trait space) and CWM values of individual traits (Fyllas 2009, Patiño et al. 2012). Traits related to drought resistance, such as wood

density, should be higher in drier sites, and traits related to nutrient acquisition and use, such as P_{leaf} and N_{leaf}, should be higher in fertile sites. We indeed found differences across sites using a multivariate analysis including the traits collected at all sites (Fig. 1B) and for most CWM traits individually (Table 4, Fig. 2). These site differences suggest that both drought and soil fertility determine CWM trait values.

The drought effect is most evident for the increase in abundance of deciduous individuals with acquisitive leaf trait values (higher SLA, lower LDMC and FPs) and the decrease in leaf area toward drier sites (Fig. 2). At drier

FIG. 2. Average community-weighted mean (CWM) leaf, stem, and whole-plant traits for different sites and two census years (black, first year; gray, last year). The sites are ordered according to increasing rainfall; dry deciduous (DD, INPA), moist semideciduous (MSD, La Chonta), moist evergreen (MEtap, Tapajós and MEpib, Pibiri), and wet evergreen (WE, Corinto) (Table 3). Fifteen traits were analyzed: (A) specific leaf area, (B) ln-transformed leaf area (measured in cm^2), (C) leaf nitrogen concentration, (D) leaf phosphorus concentration (P_{leaf}), (E) leaf N:P ratio (N:P$_{leaf}$), (F) leaf chlorophyll content, (G) leaf dry matter content, (H) specific force to punch (i.e., leaf toughness), (I) leaf mass fraction of the metamer, (J) wood density, (K) maximum diameter (DBH$_{max}$), (L) maximum crown exposure index (CE$_{max}$), (M) the percentage of individuals belonging to the Fabaceae family (% Fab), (N) the percentage of individuals with compound leaves (% Compound), and (O) the percentage of deciduous individuals (% Deciduous) (Table 1). Means and standard errors are givens. Capital letters above the bar graphs indicate significant differences between sites, and an asterisk (*) indicates significant differences between the censuses within a site. For N:P$_{leaf}$, LMF$_m$, DBH$_{max}$, CE$_{max}$ and % Fab, an interaction between site and census was found, and hence census was not significant across all sites. Sites were considered significantly different when both census years were significantly different (Appendix S9). Note that DBH$_{max}$ differed across sites in the ANOVA (Table 4), but not in the post hoc test and this figure. For statistics on effects of site, census, and the interaction between site and census, see Table 4 and Appendices S3 and S4.

sites, many species follow a drought-avoiding strategy; by being drought deciduous, they reduce water loss in the dry season, and by having small leaves, they increase heat exchange and reduce their water requirements for transpirational heat loss (Poorter and Rozendaal 2008). Drought-deciduous species have relatively short-lived leaves, and therefore invest less in structural components (e.g., low LDMC and FPs) that protect the leaves against physical damage, and more in acquisitive trait values (high SLA and N_{leaf}) to attain fast growth rates during the short growing season (Poorter 2009). High N_{leaf} may also decrease water loss in dry forests, as a high concentration of photosynthetic enzymes (that are rich in N) allows for a larger drawdown of internal CO_2 concentration in the leaf, and thus for lower stomatal conductance and water loss (Wright et al. 2001). At the wettest end of the gradient (3900 mm annual rainfall), however, SLA values strongly increase. These acquisitive trait values may be needed for efficient light capture and use in a dense forest where light is limiting tree growth and survival. Alternatively, acquisitive trait values that increase growth rates may be allowed because of the higher soil fertility at this site compared to the two moist evergreen sites.

Drought and, hence, deciduousness should affect leaf nutrient concentrations to a lesser extent than other leaf traits, as leaf nutrients can be translocated prior to leaf abscission and reused to produce new leaves (Aerts 1996, Zhang et al. 2015). Instead, soil fertility, especially phosphorus, might be a stronger driver of leaf nutrient concentrations; higher phosphorus availability (e.g., in the dry deciduous and moist semideciduous site) may increase the uptake of nutrients and the nutrient concentrations in the leaves (Maire et al. 2015), and may increase the abundance of species that can make use of high nutrient availability. The remarkably high N_{leaf} in the dry deciduous forest (INPA) is not solely explained by high soil fertility (Table 3), but also by high abundance of Fabaceae (Fig. 2K). Fabaceae species are very abundant in dry forests (Vargas et al. 2015) and have on average higher leaf nitrogen concentrations (2.79% in our data set) than other families (2.32%; Appendix S11) because of their nitrogen-fixing potential. Similarly, the relatively high N_{leaf} in the wet evergreen forest (Corinto) may be explained by the high dominance of the Fabaceae *Pentaclethra macroloba*. The almost four-fold differences among sites in leaf N:P ratio (Fig. 2E) show similar ranking among sites as P_{leaf}. The lowest N:P_{leaf} values are found at the richest site (La Chonta) that contains tracts of anthropogenic enriched *terra preta* soils with high *P* values (Quintero-Vallejo 2015). In contrast, the highest N:P_{leaf} values, indicating a relative P shortage, are found for the poorest site (Pibiri; van der Sande et al. *unpublished manuscript*) that is located on the very old and highly weathered Guiana shield (Quesada et al. 2011).

The differences between wood traits among sites indicate an effect of both rainfall and soil fertility, since sites with high WD are either low in rainfall and high in soil fertility (INPA) or high in rainfall and relatively low in soil fertility (Tapajós and Pibiri). High WD entails higher cavitation resistance, and hence continued hydraulic functioning during drought in dry forests (Markesteijn et al. 2011b). Moreover, high WD increases pathogen resistance and stem longevity (Romero and Bolker 2008), which enhances nutrient conservation on very nutrient poor soils (e.g., Pibiri; Gourlet-Fleury 2011, Baraloto et al. 2011).

We cannot fully disentangle the effects of rainfall and soil fertility, as rainfall increases and fertility decreases from southwest to northeast Amazon (Quesada 2010). However, the various CWM traits seem to be affected differently, which allows us to infer the effects of multiple environmental drivers. Using this approach, we find that rainfall most likely shapes CWM values of leaf traits associated with drought avoidance and deciduousness (e.g., SLA, FP_s), soil fertility mainly shapes leaf nutrient concentrations, the two drivers combined shape wood density, and none of the two environmental drivers determines adult stature (DBH_{max} and CE_{max}).

Old-growth forests are changing in species and trait composition

Old-growth forests are exposed to changing environmental conditions, and we therefore expected that their species composition and trait composition would change over time (cf., Enquist and Enquist 2011, Feeley et al. 2011). We indeed found significant changes in species composition over the short term (10 yr) for the three driest sites (INPA, La Chonta, and Tapajós; Fig. 3, Table 5), and over the long term (29 yr) for the site for which long-term data were available (Tapajós) (Appendix S10). The species composition seems to shift toward the center of Fig. 3, which indicates a directional convergence of plots in terms of species composition. We did not find changes in species composition for the two wettest sites (Pibiri and Corinto), possibly because wetter forests are less sensitive to changes in environmental conditions than drier forests, or simply because the number of plots in these sites (3 plots per site) was too low to detect significant changes in composition. Despite the changes in species composition for most sites, we did not find significant temporal changes in multivariate trait composition (Fig. 1A). Instead, we found significant temporal changes for individual CWM traits (Fig. 2, Table 4). Apparently, directional changes in species composition are reflected by a limited set of traits, and not by the multivariate set of traits (cf. Butterfield and Suding 2013). Focusing on multivariate strategies alone can therefore conceal important species responses to environmental change.

What drives temporal changes in trait composition?

We expected that old-growth tropical forests are affected by current changes in resource availability, drought stress, or by (historical) disturbances, and that this would cause temporal changes in CWM trait values

(Table 2). We found that WD consistently increased and SLA consistently decreased over time across all sites (Fig. 2, Table 4). Hence, both leaf and stem traits change toward a higher abundance of conservative trait values. But what is driving these changes?

Resource availability.—We expected that increased availability of resources, such as CO_2 (Appendix S4) and rainfall, would result in more acquisitive trait values rather than the more conservative trait values that we observed. Increased resource availability is therefore most likely not driving the changes in our forests. Similarly, we found no changes in nutrient concentrations and Fabaceae abundance over time (except for an increase in $N:P_{leaf}$ in the driest site and a decrease in Fabaceae abundance in Tapajós). Therefore, increased nitrogen deposition (cf. Hietz et al. 2011) is not a likely driver of the changes we observed.

Drought.—The increase in conservative trait values could be the result of increased (atmospheric) drought and/or temperature stress (Enquist and Enquist 2011, Feeley et al. 2011). We did not observe a consistent decrease in annual rainfall or increase in rainfall seasonality (Table 3; Appendix S5) or in drought (Appendix S6) in our sites, but atmospheric drought stress also depends on changes in factors such as temperature and drought events, and drought events have occurred repeatedly since 1900 (Appendix S6). With increasing temperature, we would expect a decrease in leaf area and also a decrease in the abundance of species with compound leaves, as small leaves or leaflets facilitate heat exchange (Poorter and Rozendaal 2008), but we did not find such changes (Fig. 2B,N). With an increase in drought, we would expect an increase in the abundance of drought-avoiding deciduous species, which we did not find (Fig. 2O). Moreover, we would expect a reduction in potential adult stature (indicated by DBH_{max}), as tall species have more exposed crowns and longer hydraulic path lengths, which makes them more prone to hydraulic failure under drier conditions (Phillips 2010, Bennett et al. 2015). We indeed found a tendency for a significant temporal decrease in DBH_{max} for the two wettest sites, but a tendency of DBH_{max} to increase in the three driest sites. Possibly, wet forests suffer more from drought than dry forests, although the safety margins to cavitation are rather similar for wet and dry forest trees (Choat 2012), and our wettest forests have experienced increasing rather than decreasing rainfall patterns (Table 3; Appendix S6). Furthermore, the reduction in percentage of Fabaceae trees in moist evergreen forest (Tapajós) in the short term (Fig. 2) and long term (Appendix S10) indicates no increased drought stress, as Fabaceae species are generally more drought tolerant and more abundant in dry forests (Adams et al. 2010, Vargas et al. 2015). Alternatively, drought is affecting other aspects that we did not measure, such as rooting depth. Nevertheless, a lack of trend in the percentage of deciduous trees, an increase in

DBH_{max} in the driest sites, and no increase in Fabaceae abundance, suggest that compositional changes are not due to increased drought stress. Hence, although we cannot fully exclude an increased drought stress on a longer timescale, it seems not to be the main driver of changes in species and trait composition in our forests.

Disturbances.—It is most likely that these forests are undergoing a successional change from early-successional, light-demanding species with high SLA and low WD toward a higher abundance of late-successional, shade-tolerant species with lower SLA and higher WD (Poorter et al. 2006, van Gelder et al. 2006). Most observed trait changes are in line with what we expected when forests recover after disturbances (Table 2). The decrease in percentage of Fabaceae individuals in moist evergreen forest (Tapajós) suggests, for example, a successional change toward older forests, which are generally less N limited (Batterman et al. 2013, Sullivan et al. 2014). This decrease in N limitation is further supported by an increase in the $N:P_{leaf}$ for dry deciduous forest (INPA; Fig. 1E). Possibly, the forests are still recovering from past disturbances. After disturbance, forest structure and species richness recover relatively fast (e.g., de Avila et al. 2015, Poorter et al. 2016), but many tropical tree species can live for hundreds of years (Chambers et al. 1998), and therefore the recovery of species composition, and hence functional trait composition, can take more than a century for temperate forests (Vellend et al. 2006), and probably even longer for some tropical forests (Chazdon 2003). We found no relationship between changes in trait composition and changes in biomass (Appendix S12), which suggests that the successional changes in trait composition in our sites do not result in a change in forest structure, possibly because forest structure recovers faster than trait composition (Martin et al. 2013).

Many recent studies show that old-growth Neotropical forests are not pristine, but disturbed by pre-Columbian (Heckenberger et al. 2003, Clement et al. 2015) or more recent human occupation (Redford 1992, van Gemerden et al. 2003). For example, for one of our sites (La Chonta), the presence of *terra preta* soils suggest that it had been occupied by indigenous people a long time ago (Quintero-Vallejo et al. 2015). Recovering from other disturbances is also possible, such as intense (El Niño) drought events (as opposed to a long-term increase in atmospheric drought stress), large-scale and intense fires, and wind storms (Nelson et al. 1994), which are all frequently observed across the Amazon (Nelson 2005). Disturbance events lead to canopy tree dieback and more light availability in the understory (Nepstad et al. 2007, Phillips 2010). During initial recovering from such events, we would expect to see an increase in the abundance of light-demanding species with low WD and high SLA (Carreño-Rocabado et al. 2012, Karfakis and Andrade 2013), but during later phases of recovery when light availability reduces, the abundance of shade-tolerant

species with high WD and low SLA should increase. Such patterns of successional change could also be observed as an artefact due to small plot size and distribution of plots (Fisher et al. 2008). When natural disturbances (e.g., tree-fall events) are of a similar size as the plots, then the chance is high that these events will not occur during the census period. Instead, it is then more likely to sample plots that were disturbed before the census period, and that are thus undergoing successional change. However, most natural disturbance events occur on small spatial scales (<0.1 ha; Jans et al. 1993, Espírito-Santo 2014), and we therefore expect that our plots of 0.25–1 ha well represent the heterogeneity in forest dynamics and structure (Chave et al. 2004), and thus that this possible artefact cannot explain the successional changes in species and trait composition across our forests.

The type of disturbance responsible for the observed changes in species and trait composition should have a relatively low intensity and/or have occurred many decades to centuries ago, as these old-growth forests seem to be in late phases of recovery. Given the consistent changes in composition across the five forests, recovery from disturbance events that occur regularly across the Neotropics, such as El Niño droughts, are more likely to explain the observed changes in trait composition than local-scale disturbances that do not occur across the Neotropics, such as wind storms and fire. All sites have experienced frequent drought events since 1900 at different moments in time (Appendices S5 and S6), and some of these may have caused considerable disturbance to the forest. Hunting pressure could also change the species and trait composition, but this would decrease the dispersal of large seeds and the abundance of large-seeded and late-successional species (Foster and Janson 1985, Galetti 2013), and can thus not explain our results. An alternative explanation for the observed successional patterns is that in the past, anthropogenic disturbances by rural people were more widespread. With a recent migration of rural people to urban areas, this pressure has been released, leading to forest recovery (Wright 2005).

For the longer-term temporal changes (29 yr) in a moist evergreen forest (Tapajós), we found an increase in DBH_{max} and WD (Appendix S10), supporting the successional change that we found across all sites for a shorter time period. Although SLA did not change, we found a decrease in LA, possibly because late successional species have on average small or intermediate-sized leaves (Poorter and Rozendaal 2008). Moreover, the abundance of individuals of Fabaceae decreases over this long-term period, which supports our hypothesis that a gradual increase in drought stress is likely not the main driver of change.

CONCLUSIONS

Even over relatively short time scales (10 and 29 yr), we find consistent changes in species and trait compo-

sition. The shifts in functional composition across the sites suggest that not only the species and trait composition, but also the ecosystem processes are changing, with lower SLA and higher WD leading to slower carbon sequestration, longer-term carbon storage and "slower" forests (Finegan 2015). A recent analysis of three decades of carbon dynamics in Amazonian forest plots also shows that these forests are slowing down in carbon sequestration (Brienen 2015). The authors suggested that this slowing down of carbon sequestration is caused by higher CO_2 concentrations leading to a speeding up of the life cycle of trees, and a faster tree turnover. For our old-growth forests, however, we find that the slowing down of the forest is most likely explained by successional forest recovery from disturbances that occur regularly across the Neotropics (e.g., El Niño droughts).

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1890/15-1815.1/suppinfo

Data Availability

Data associated with this paper have been deposited in Data Archiving and Networked Services (DANS): http://dx.doi.org/10.17026/dans-xd8-37qq