# Functional ecology of secondary forests

# in Chiapas, Mexico

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AV2010-19

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Functional Ecology of Secondary forests in Chiapas, Mexico

Master thesis Forest Ecology and Forest Management Group

FEM 80439 and FEM 80436

April 2010, AV2010-19


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## General introduction

Secondary and degraded forests are increasing in surface and importance in wet tropical regions (Guariguata & Ostertag 2001) and should be included in conservation strategies when taking a more integrated view on tropical landscapes (Letcher & Chazdon 2009). Secondary forests are forests that have naturally regenerated after complete clearance of the vegetation through anthropogenic disturbance. What processes are underlying natural regeneration on abandoned agricultural land has been a key challenge in ecology and their study has become increasingly relevant in times of global change in climate and land-use (Naeem & Wright 2003).

In our study location in Chiapas, Mexico (Figure 1) secondary forests are largely part of swidden fallow or shifting cultivation systems, which are common throughout the tropics and one of the major factors causing deforestation (*e.g.* Fox *et al.* 2000). Shifting cultivation is the dominant cause of deforestation in tropical Mexico (Turner *et al.* 2001) and 48% of deforestation cases are associated with this type of land use worldwide (Geist & Lambin 2002). Although shifting cultivation of maize in Mexico (the 'milpa') is appreciated for the multiple use of tropical forest where crops and non-food crops from secondary forests are sequentially harvested (Alcorn and Toledo 1998), the sustainability of this land-use type depends on underlying economic, institutional, political, social and demographic forces which vary across regions. Though shifting cultivation has been practiced for over centuries, it has become increasingly unsustainable due to rapid expansion by clearance of old-growth forest and intensification by reducing the fallow period (*e.g.* Geist & Lambin 2002; Toledo *et al.* 2003).

Studying secondary succession after abandonment of the cultivated fields deals with forest recovery after clearance. During secondary succession the structure of the vegetation, the local environmental conditions and the vegetation composition change. Predictable shifts in selected functional types occur where some functional traits replace others during this process (*e.g.* McKinney & Lockwood 1999; Grime *et al.* 2000; Díaz *et al.* 2007). Functional traits are physical attributes caused by genetic expression which serve as indicators or estimators of plant responses to environmental factors (Lavorel & Garnier 2002; Cornelissen *et al.* 2003) and their value and range are thought to be important contributors to ecosystem processes (Tilman 2001). Because secondary succession can be described as ongoing community assembly, it provides a suitable background against which functional composition of communities can be analyzed to attain information on drivers of community assembly and trade-offs in species' functional strategies. Understanding the functional ecology of secondary succession is relevant to be able to conclude on the effect of global change drivers (agricultural expansion, intensified shifting cultivation) on ecosystem functioning and ecosystem services or when management priorities seek to manipulate species composition (Loreau *et al.* 2001). Current master thesis focuses only on part of this issue by deepening our insights into forest recovery after shifting cultivation and driving forces behind natural regeneration. This is done through studying patterns of changing functional composition during secondary succession after deforestation for maize cultivation by unraveling stand characteristics, environmental gradients, species composition, functional traits and functional diversity. Present study contains in depth studies on the following four aspects (chapter 1 - 4) which are written as independent articles.

**1. Environmental filtering of functional traits as a driver of community assembly during secondary succession in tropical wet forest of Mexico**

We analyzed major changes in community composition during secondary succession with a stepwise approach; fallow age influencing stand basal area, which influences understory environmental conditions which in turn explain community functional trait composition.

**2. Functional traits related to changing environmental conditions during secondary succession: Environmental filtering and the slow-fast continuum**

We tested the slow- fast (growth- survival) trade off in secondary forest species' functional strategies and the environmental filtering hypothesis by testing for consistent trait-environment linkages along a successional gradient.

**3. Functional and species diversity in tropical wet forest succession**

We studied functional diversity changes along secondary succession and how functional diversity relates to species diversity.

**4. Functional diversity as a tool in predicting community assembly processes**

We used functional diversity as a tool to analyze whether environmental filtering or competition selects few of the species present to become dominant and how this changes with succession.
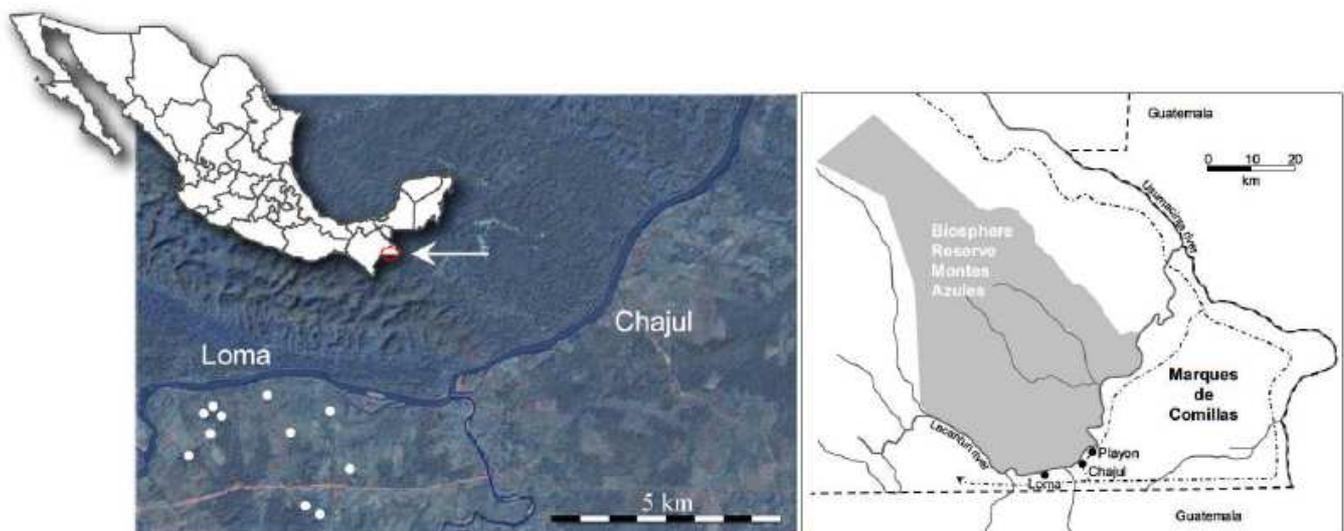


*Figure 1: General overview of the study area in Chiapas, Mexico. The study sites are secondary forests around the village of Loma Bonita, close to Chajul, south of the river the Lacantún. North of the river is the Montes Azules biosphere reserve. The white dots indicate the locations of the permanent sample plots studied (Modified from Breugel 2007).*

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# Chapter 1: Environmental filtering of functional traits as a driver of community assembly during secondary succession in tropical wet forest of Mexico

**Abstract**

Environmental filtering is seen as one of the major selective forces shaping community assembly, implying that community membership is restricted to those species that can reach the site and establish under the specific environmental conditions.

In this study we tested the environmental filtering hypothesis, analyzing forest development in terms of stand structure, subcanopy environmental conditions and functional traits for a large set of species along a chronosequence in tropical wet forest of Mexico.

As hypothesized, we found that with fallow age the stand structure and environmental conditions change directionally and that variation in plant functional traits is related to these environmental changes. This strongly indicates that environmental filtering shapes plant communities. Furthermore this study shows that habitat filtering takes place at different levels of community assembly, one level determining species presence and another shaping species dominance, and distinct environmental conditions are found to explain these aspects. Light availability influences presence of species with certain traits whereas air temperature and soil water contents determine successfulness (abundance) of traits. If indeed ecosystem functioning is largely determined by the dominant species, as predicted by Grime's biomass ratio hypothesis, these findings suggest major effects of climate change on functioning of tropical forests.

Keywords: Secondary succession; Stand structure; Environmental conditions; Functional traits; Community assembly; Environmental filtering; Chronosequence; Mexico; Wet tropical forest

**Introduction**

Over 50% of the world's forests are modified natural forests; forests of naturally regenerated native species with clearly visible indications of human activity (FAO 2005). Secondary and degraded forests are increasing in surface and importance in wet tropical regions throughout the world and should be included in conservation strategies when taking a more integral view of tropical landscapes (Letcher & Chazdon 2009). What processes are underlying natural regeneration on abandoned agricultural land has been a key challenge in ecology and their study has become increasingly relevant in times of global change in climate and land-use (Naeem & Wright 2003).

A mechanistic explanation of community assembly states that community membership is restricted to those species that are able to reach the site and establish under the specific environmental conditions (Belyea & Lancaster 1999). This theory predicts that functional profiles (certain sets of functional traits) along gradients are shaped by the process of environmental filtering; a hierarchical set of filters removing species which lack traits (morphological, physiological and ecological) for persistence under particular circumstances. Examples of major environmental filters that determine which traits and functions can persist at a given site are climate, disturbance regime, soil and atmospheric conditions (Keddy 1992; Díaz *et al.* 1998, 2007).

The environmental filtering theory has been tested on a gradient of forest development. As the forest develops community structure, environment and species composition change over time (Lebrija-Trejos *et al.* 2010). Secondary succession is studied using age as an independent factor influencing stand structure, which in turn alters the environmental conditions that filter for functional traits persisting at a site. This stepwise approach is based on previous findings that community structure drives succession, as opposed to fallow age per se which is more a compound variable reflecting community organization at a certain point during succession (Breugel *et al.* 2006).

Chronosequence studies on tropical forest succession invariably show that basal area increases with fallow age (*e.g.* Saldarriaga *et al.* 1988; Brown & Lugo 1990; Peña-Claros 2003; Chazdon *et al.* 2007), albeit at very different rates (Guariguata & Ostertag 2001). A study in the same region also showed basal area to increase in early stages of succession due to growth effects overriding effects of recruitment and mortality (Breugel *et al.* 2006). Stand density is expected to decrease with time as biomass will be accumulated in fewer but bigger individuals (*e.g.* Brown & Lugo 1990), however density may show unclear patterns due to highly variable patterns of mortality and recruitment between sites in chronosequence studies (Chazdon *et al.* 2007).

Stand structure influences light availability, something that is extensively studied, especially in the light of tree seedling regeneration (*e.g.* Denslow *et al.* 1990; Palik *et al.* 1997; Nicotra *et al.* 1999). Lebrija-Trejos *et al.* (2010) showed that as the structure of the woody vegetation changes the understory environmental conditions change in Mexican dry forest, where Photosynthetic Photon Flux Density, air temperature, soil temperature and soil water potential were assessed. In the present study we assessed the variables light availability, air temperature, air relative humidity, soil water contents and soil temperature in their relation to stand basal area and stand density. The relation between stand structure and understory environmental conditions is best described by changes in basal area. Tree basal area is an accepted predictor of foliar biomass and with that of leaf area index (*e.g.* Kendal Snell & Brown 1978; Burton *et al.* 1991). Because increased leaf area index is directly related to the light available in the understory (Beer-Lambert law), increased leaf area index reduces understory temperatures and is expected to reduce evaporation causing increases in the relative humidity and soil water content. This in line with previous study results in the same area where air and soil temperature, canopy openness and Photosynthetic Active Radiation decreased during secondary succession whereas soil water contents increased (Ricaño-Rocha 2007).

Plants are functionally adapted to grow under specific environmental conditions. Through the process of habitat filtering specific functional types are selected from the regional species pool (*e.g.* Diamond 1975; Keddy 1992). Even though correlation does not prove causation, broad patterns of trait-environment relations have been described and used as an indication of the effect of environmental filtering. Functional traits adaptations related to successional status are based on a trade-off in plant design that allows fast growing types with a rapid acquisition of resources in the high light early stages of succession, and slow functional types that permit conservation of resources in late successional stages where light has become limiting. For example, early successional species have a high leaf area and low wood density to attain high resource capture and rapid growth, whereas late successional species conserve internal resources and grow more slowly, characterized by high wood density and tougher leaves (Bazzaz & Picket 1980; Reich *et al.* 2003; Poorter *et al.* 2008). This slow-fast continuum is shown to be maintained across different geographic regions and

consistent across floras and phylogenetic groups (Díaz *et al.* 2004), and is also referred to as the growth-survival trade-off (*e.g.* Kitajima 1994; Sterck *et al.* 2006; Poorter & Bongers 2006).

The stepwise approach (age- stand structure- environment- traits) allows studying functional traits in the context of abiotic gradients which have been indicated a priority in previous studies (*e.g.* Niinemets 2001; Wright *et al.* 2005; McGill *et al.* 2006). Furthermore, analyzing the importance of environmental conditions in explaining variation in functional traits addresses the role of environmental filters in community assembly in secondary forests in the study region.

This study aims at determining the major changes in community structure during secondary succession. It specifically addresses the questions:
1. How does stand structure of secondary forests change after time since abandonment?
2. How do environmental conditions change with changes in stand structure?
3. Is the change in community level functional trait composition of the sapling stage related to changes in environmental conditions?

We hypothesized that:
1. After the agricultural field has been abandoned stand density will decrease in our chronosequence of sites and stand basal area will increase in early secondary succession.
2. With increased aboveground biomass, less light is available in the understory and the air and soil temperatures will drop, resulting in decreased evaporation and increased humidity of the air and soil water contents.
3. Understory environmental conditions explain variation in trait composition of saplings observed between secondary forest sites indicated by strong relationships between trait abundances and environmental conditions.

## Methods
*Research site*
The research location for this study is near the Chajul tropical field station, south of the Montes Azules Integral Biosphere Reserve, at the border of the Marques de Comillas region, within the Lacandon region in southeast Mexico (16°01'N, 90°55'W). The climate is warm-humid with an average annual temperature of 25-27°C and an annual rainfall of 3.000 mm, with less than 100 mm per month falling in the dry season (February-April) (Ibarra-Manríquez & Martínez-Ramos 2002; Breugel *et al.* 2006). The study takes place around the village of Loma Bonita, an area characterized by small hills and valleys with sandy or limestone soils of low pH (<5.5). Currently 65% of the land area around Loma Bonita is dedicated to agricultural practices (mostly livestock pasture), 14% is mature forest and 21% consist of secondary forests, of different ages. The old growth forests around Loma Bonita have suffered extensive pressure for changing land-uses, even more so than surrounding villages, possibly due to colonization of refugees from Guatemala since '82, giving rise to the current large area of secondary forests (Zermeño-Hernández 2008).

The chronosequence consists of 11 secondary forest sites (10 x 50 m) with fallow ages between 3.5 and 22.5 years (plot 1-11 reflect increasing fallow age). All sites are established on abandoned corn fields, the main form of agriculture, and plots were in use within one year before abandonment.

*Species selection*
This study focuses on saplings (woody plants with a DBH > 1cm and between 1.30 and 3 m high). Saplings are chosen because of the relative ease of measuring functional traits and saplings are expected to be strongly affected by filtering forces while trying to reach the upper canopy, as opposed to canopy trees which are largely a product of past filtering processes.
For each species the relative importance value is calculated based on the relative number of stems and the relative basal area. It is calculated as: $(BA_i/BA_{total} + D_i/D_{total})/ 2$.
Where $BA_i$ is the basal area of the $i^{th}$ species, $D_i$ is the density of the $i^{th}$ species and total reflects the total of individuals in the selected sapling category.
The summed importance value of selected species makes up for at least 80%, where dominant as well as rare species have been included. In total 82 species are selected; an average of 20 species per site, ranging from 7 to 32 with an average importance value of 0.05 (5%), ranging from 0.002 (0.2%) to 0.89 (89%).

*Forest development*
Different aspects of forest development after abandonment of agricultural fields have been studied; age, stand structure and environmental characteristics.
The fallow age of the secondary sites is determined based on information of landowners and other local residents (Breugel *et al.* 2007). Stand basal area ($m^2$/ha) and stand density (#/ha) are calculated based on recorded data on al individuals (DBH > 1cm).
Sub-canopy environmental variables were measured during the dry season (March and April). Air temperature and relative humidity were measured in the vegetation (height of 1.5m) by using HOBO temperature / relative humidity data loggers (HOBO Pro v2 Temp/RH logger, Onset Computer Corporation, UK). For each of the 10 HOBO dataloggers a measurement was taken every minute for 15 minutes. The percentage of canopy openness (here measured as an indicator of light availability) is measured with a spherical densiometer (Spherical Densiometer Concave). A densiometer is sometimes criticized for not being a very accurate measure of light conditions (*e.g.* Engelbrecht & Herz 2001), but sites showed very distinct levels of light availability (ranging between 15% to 70% of openness or light availability) and the relative simplicity of the canopy in secondary sites compared to old growth forest (*e.g.* Brown & Lugo 1990) legitimizes the use of this method. Also two independent days of sampling have been used to compromise for accuracy, all measurements taken by the same person. Soil temperature (°C) and water content (% volume) were assessed with a W.E.T. Sensor (type WET-2, Delta-T Devices Ltd) at a depth of about 10 cm.
The environmental variables have been measured for 3 days (2 days in case of densiometer measurements) during the dry season, at least 3 days after the last rain, on days without overcast. Dates measuring subcanopy environmental conditions are randomly spread over the sampling period. Measurements were carried out at the hottest and driest part of the day, between 13.00 and 16.00. This is expected to capture the most extreme conditions to which plants need to be adapted and considered a good way to illustrate differences

between the sites. Per plot 10 replicates were taken at even distances in a transect of 50 meters at the center of the plot. (Site 3 only allowed 4 replicate measures instead of 10 because it had partly been cleared.)

*Functional traits*
Nine functionally important traits (Table 1; for methods see Chapter 2) were studied and scaled up to the community level by the Community Weighted Mean (CWM) (Díaz *et al.* 2007; Lavorel *et al.* 2007), a parameter that can be seen as the functional value of a randomly sampled plant from the community.

It is calculated as: $\quad CWM = \sum\limits_{i=1}^{s} W_i \, x_i$

Where S is the total number of species, $W_i$ is the relative importance of the ith species and is the trait-value of the ith species.

*Table 1. Leaf trait abbreviations, a short description of their functional role and the references used.*

| Trait abbreviations (units) | Functional role | References |
|---|---|---|
| Leaf area; LA ($cm^2$) | Light intercepting area, dry matter production, respiration, leaf cooling, gas exchange | Bazzaz & Picket 1980<br>Popma *et al.* 1992 |
| Specific Leaf Area; SLA ($m^2$ $Kg^{-1}$) | Light capture economics, net assimilation rate, relative growth rate, leaf life span, photosynthetic capacity | Reich *et al.* 1997<br>Poorter & Garnier 1999<br>Sterck *et al.* 2006<br>Poorter & Bongers 2006 |
| Leaf Dry Matter Content; LDMC ($g\ g^{-1}$) | Leaf tissue density, level of assimilative compounds and transfer conductance to $CO_2$, construction costs and nutrient retention, against mechanical and herbivore damage, tolerance against water limitations | Poorter & Garnier 1999<br>Niinemets 2001<br>Garnier *et al.* 2004 |
| Leaf Thickness; LT (mm) | Construction costs, leaf life span, photosynthetic rates per unit leaf area, against mechanical and herbivore damage, gas exchange and leaf cooling | Reich *et al.* 1991<br>Popma *et al.* 1992<br>Niinemets 2001<br>Díaz *et al.* 2004<br>Onada *et al.* submitted |
| Specific Force to Punch; sFtP (MN $m^{-2}$) | Leaf strength, survival of herbivory and physical damage, persistence, leaf lifespan | Coley 1983<br>Poorter & Bongers 2006<br>Poorter *et al.* 2008<br>Onoda *et al.* submitted |
| Petiole length; PL (cm) | Light capture efficiency | Black 1960<br>Takenaka 1994 |
| Instantaneous chlorophyll fluorescence; | Ability to dissipate heat, resistance against overheating, respiration, photosynthetic performance | Maxwell & Johnson 2000<br>Baker & Rosenqvist 2004 |

| | | |
|---|---|---|
| $F_0$ (no unit) | | |
| Quantum Yield; QY (no unit) | Effectiveness of photosystem II, $CO_2$ assimilation, stress tolerance, photosynthetic performance, leaf nutrient levels | Maxwell & Johnson 2000 Parkhill *et al.* 2001 Baker & Rosenqvist 2004 Dos Santos *et al.* 2006 |
| Wood Density; WD (g cm$^{-3}$) | Construction costs, growth rate, stem vulnerability, mortality rate | Sterck & Bongers 2001 Poorter *et al.* 2008 Chave *et al.* 2009 |

*Statistical analysis*

Stand structure variables were calculated based on the basal area and number of individuals of all woody species monitored for each of the sites (DBH > 1cm) and recalculated in per-hectare values for better comparability with other studies. Environmental variables are average values of the three different days (two in case of light availability), ten replicate measures per site and in case of the HOBO data loggers also across the 15 measurements (each minute). This resulted in one value per site, reflecting average subcanopy environmental conditions at midday during the dry season. Functional traits have been scaled up to one average value per site by the simple community mean (based on presence/absence of species) and by the Community Weighted Mean (weighted by species abundances per site).

The stepwise approach to major structural changes during succession is analyzed in a pathmodel (age affecting stand structure, stand structure affecting understory environment and environmental conditions affecting community assembly based on trait composition). The relations of the pathmodel were assessed using linear regression and multiple linear regression (backward analysis). For the variables selected by the multiple linear regression, an additional curve estimation was carried out for clearer description of the successional changes. Principal component analysis has been used to separate sites based on differences in subcanopy environmental conditions and on mean community trait-values (simple and weighted for species abundances). Not normally distributed data was transformed to improve parametric model fit. SPSS 17.0 was used for all analysis.

**Results**

Major changes in stand structure, environmental conditions and functional trait composition take place during secondary succession (Figure 1). Stand density does not significantly relate to time after abandonment in secondary forests (R= .325; p= .288) (Figure 2a). Stand basal area increases with time, where the relation is not significantly linear (R= .568; p= .068) (Figure 1) but better described by an inverse model ($R^2$= .588; p= .006)(Figure 2b).
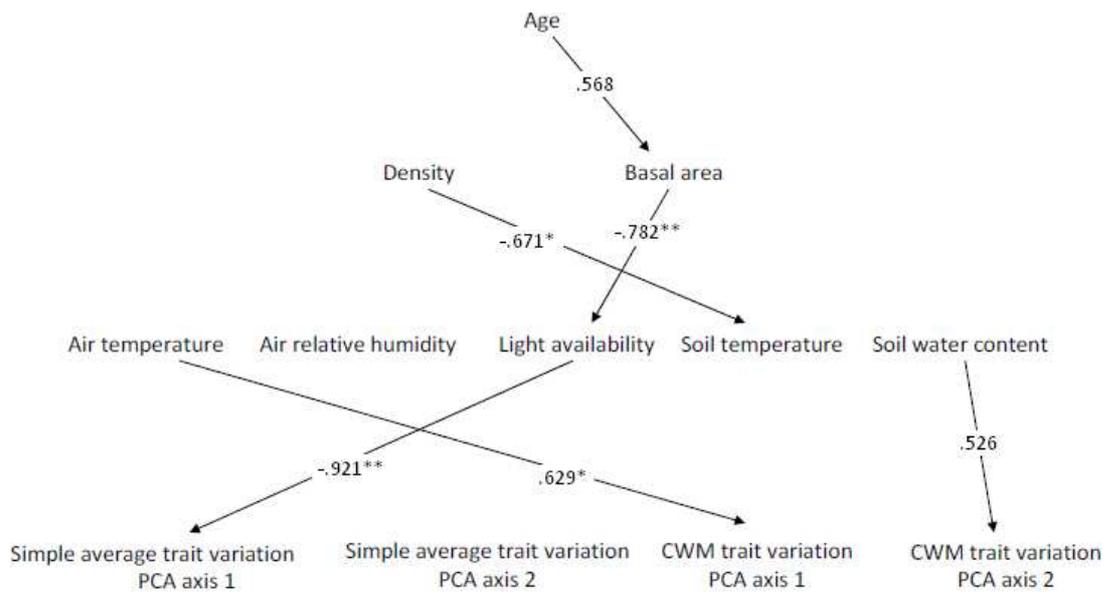
*Figure 1: Pathmodel showing the causal relations between fallow time, stand structure variables (density and basal area), subcanopy environmental conditions (air temperature, air relative humidity, light availability, soil temperature and soil water content) and the community-level trait variation among the sapling communities (simple average trait variation PCA component 1 and 2 and community weighted mean trait variation PCA component 1 and 2) in the secondary forest plots. Arrows signify relations selected by a backward multiple linear regression (\* p < .05, \*\* p < .001).*



a.                                                                                    b.
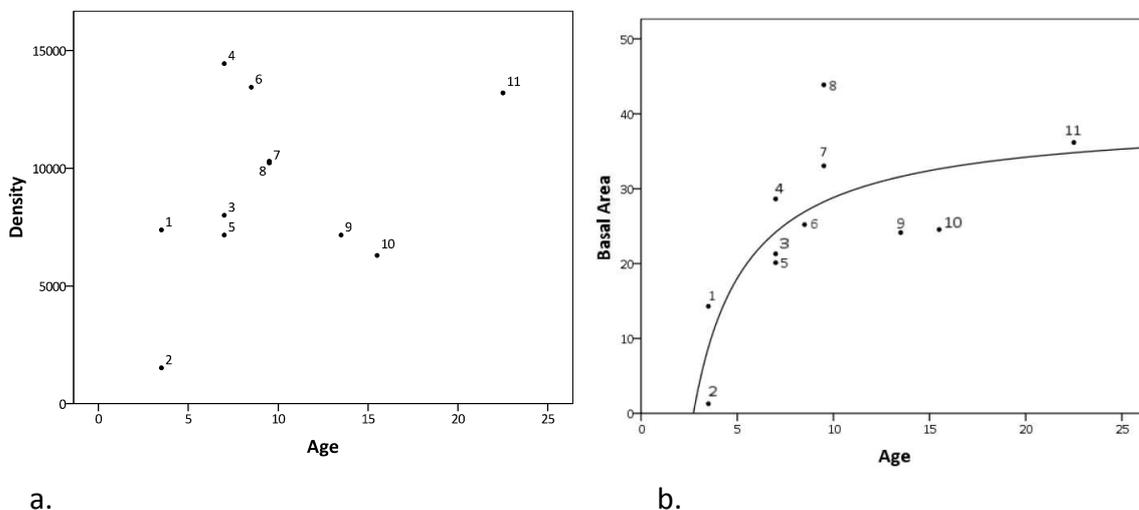
*Figure 2.  Structural characteristics of the eleven secondary forest sites (age 3.5-22.5 years) against time after abandonment. a. stand density (#/ ha) shows no significant relationship, b. stand basal area (m$^2$/ ha) fitted the inverse model (R$^2$= .588; p= .006).*

The sites studied vary in sub-canopy abiotic conditions, 82% of the variation among plots is explained by the first two principal components axes based on site differences in air temperature, air relative humidity, light availability, soil temperature and soil water content (Figure 3). The main component of this variation is strongly correlated to the age of the sites, reflecting the inverse model (R$^2$= .653; p= .003) (Figure 4).
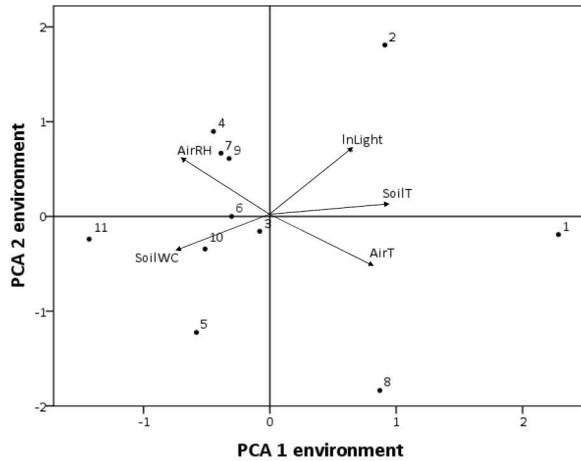
*Figure 3. The secondary forest sites separated based on the subcanopy environmental conditions (air relative humidity, air temperature, light availability, soil water content, soil temperature). The first axis explains 58% and the second axis 24% of the environmental variation between the sites.*
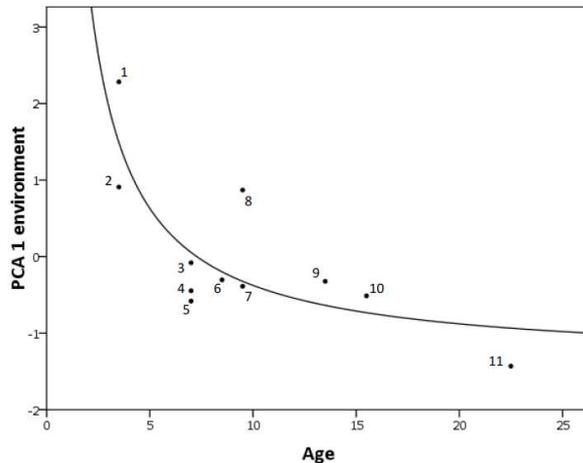


*Figure 4. The first component of the PCA (58%) separating sites based on their subcanopy environmental conditions is significantly explained by age ($R^2$= .653; p= .003).*

Relating the subcanopy environmental conditions to the stand structure variables density and basal area with a multiple linear regression we found that light availability (ln transformed) depends largely on the stand basal area (R= -.782; p= .004)(Figure 1). This relation is best described by an exponential model ($R^2$= -.746; p= .001) (Figure 5a). Sites with a higher stand basal area have less light penetrating into the sub-canopy environment. Soil temperature is significantly explained by stand density (R= -.671; p= .024) (Figure 1), reflecting a linear model ($R^2$= .450; p= .024) (Figure 5b) where increased density reduces the temperature. Soil water contents, air relative humidity and air temperature are not explained by the stand structure characteristics measured (Figure 1).
The environmental conditions measured are not independent (Pearson correlations: lnLight – Soil temperature R= .706; p= .015 Soil temperature- air temperature R= .754; p= .007 air relative humidity- air temperature R= -.713; p= .014).

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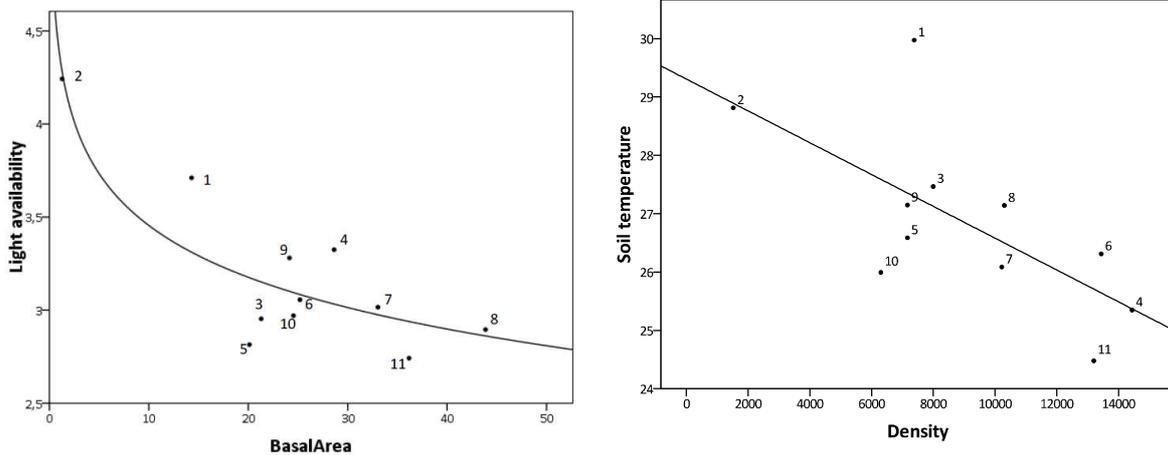
a.                                                                    b.

*Figure 5. Stand structure variables explaining the changes in environmental conditions. a. Light availability (ln transformed) in the sites depends on the stand basal area, best described by an exponential model ($R^2$= -.746; p= .001). b. Soil temperature is significantly explained by stand density with a linear model ($R^2$= .450; p= .024).*

75% of the variation in plant trait presence (simple community average) among sites is explained by the two component axes (Figure 6a). This variation is related to abiotic conditions. Multiple regression analysis shows that the scores of the first axis can be explained by light availability (R= -.921; p= .000) (Figure 1) confirming the linear model ($R^2$= .848; p= .000)(Figure 7) while the other environmental variables have no effect. The second axis is not significantly related to any of the environmental variables studied.

Taking into account the dominance of traits in the communities (community weighted mean), 63% of the variation between sites is explained by the first two component axes in the PCA (Figure 6b). The scores of the first axis can be explained by air temperature (R= .629; p= .038)(Figure 1) following a linear model ($R^2$= . 396; p= .038)(Figure 8a). For the second axis soil water content is selected by the multiple linear regression as explaining variable (R=.526; p=.096)(Figure 1) although the relation is not significant ($R^2$= .277; p= .096) (Figure 8b). The two PCA graphs based on presence/absence and based on trait dominance show a complete different segregation of sites, the PCA axes of both graphs are not correlated (Pearson, results not shown).
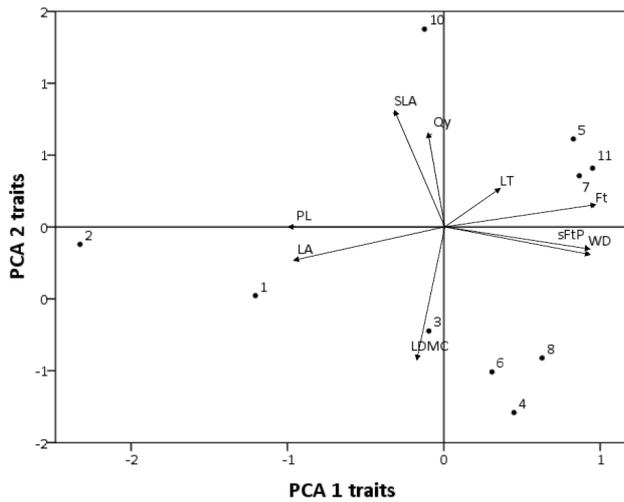
a.
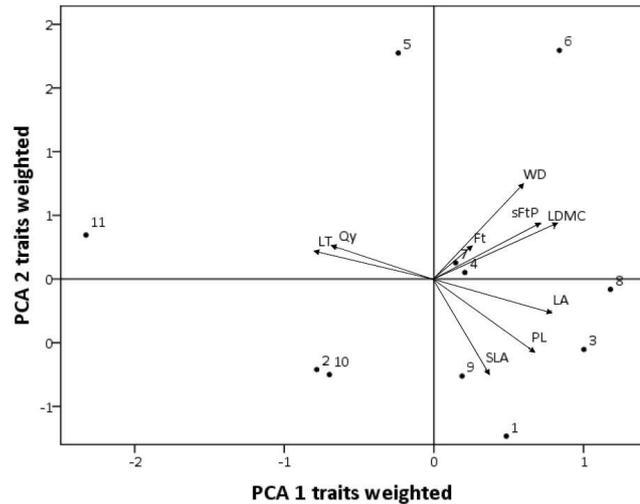


b.

*Figure 6a. PCA separating the successional plots based on trait value presence (simple community average). PCA component 1 (52%) can be explained by light availability and the second axis (23%) is not significantly related to any of the environmental variables studied. b. PCA separating the successional plots based on weighted trait values (community weighted mean). The scores of the first axis (42%) can be explained by variation in air temperature and the second axis (21%) is related to soil water contents.*
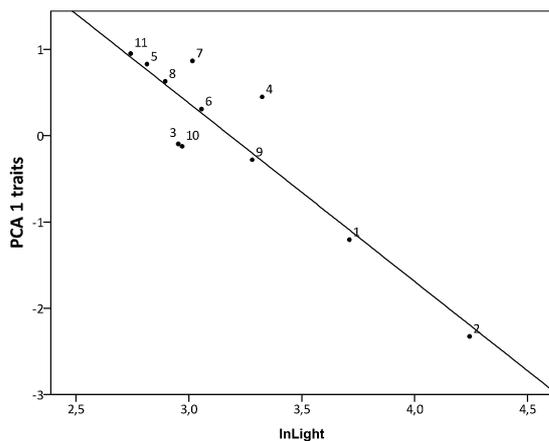


*Figure 7. Light availability strongly explains the first component axis of the PCA where plots are separated based on simple trait averages ($R^2$= .848; p= .000).*
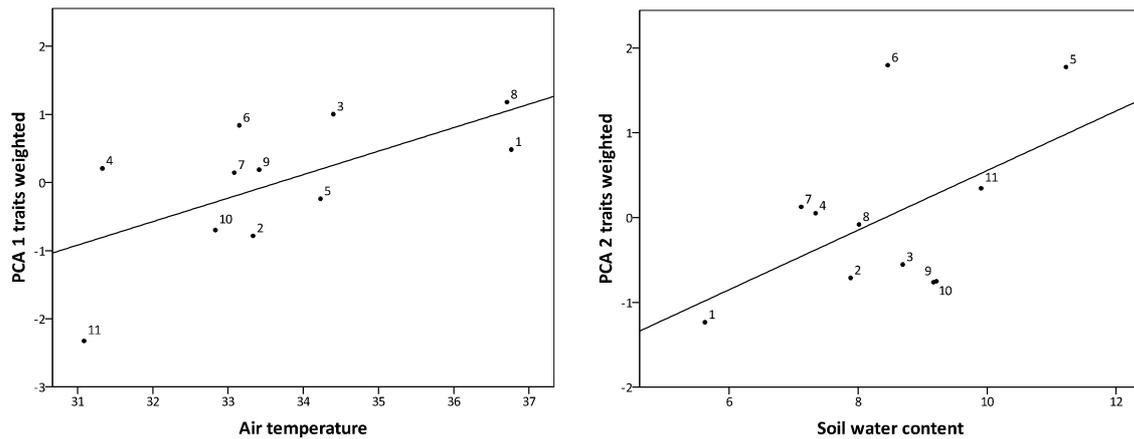
*a.* *b.*

*Figure 8a. The first principal component of the PCA separating sites based on community weighted mean trait values is explained by air temperature ($R^2$= .396; p= .038). b. The second axis of the principal component is explained by soil water content ($R^2$= .277; p= .096), although the relation is not significant.*

## Discussion

The stepwise approach taken in this study to identify major changes in community structure during secondary succession is illustrated in the path model (Figure 1).

Stand structure changes with time after abandonment, although no significant results are found for stand density. Stand density was hypothesized to decrease due to increased thinning effects in time. Our results show unpredictable changes in number of stems in the chronosequence, as was shown before in the same region (sites up to 17 years after abandonment) (Chazdon *et al.* 2007). Density is the result of recruitment, growth and mortality and is likely influenced by a range of factors acting upon different temporal and spatial scales and which vary in their effects on different size classes (Breugel 2007a). For example, Breugel (2007b) showed high mortality effects for one pioneer species (*Cecropia peltata*) compared to another (*Trichospermum mexicanum*) in the same study area. Mortality of *Cecropia* was stronger for smaller individuals, and they explain the mortality differences between the pioneer species by differing tolerance to neighbourhood competition. This example illustrates that massive mortality effects of early pioneer species are common in the study area and this can explain the variation in stand density among sites and the lack of stand density pattern in the chronosequence. Basal area, in contrast, increases with age following the inverse model (Figure 2b), probably because the larger individuals are less prone to neighbourhood competition. This saturating increase in stand basal area is a well established in studies on secondary succession and shown by several previous studies (*e.g.* Brown & Lugo 1990; Breugel *et al.* 2006).

Generally, variability in stand structure among sites in our chronosequence may be because younger sites tend to vary more due to influences by factors other than age, like factors in the landscape matrix such as proximity to seed sources (Chazdon *et al.* 2007). Also, our chronosequence is rather short (3.5 to 22.5 years of fallow age) and is poorly replicated especially at the extremes of the chronosequence which will restrict the finding of clear patterns.

Environmental conditions show directional changes with variation in stand structure, as hypothesized. With increasing stand basal area during forest development, light availability decreased exponentially (Figure 5a), as has been shown earlier (*e.g.* Palik *et al.* 1997). At the same time, with increasing stand density the soil temperature dropped (Figure 5b). This is against expectations, as we expected density and soil temperature both to decrease with age. This result is hard to explain in terms of succession because of the idiosyncratic patterns of density with fallow age, suggesting that density is important determining environmental conditions though largely independent of the gradient of succession. Results of a study in Mexican dry forest also showed that environmental conditions were explained by stand structure characteristics. Here stand density explained soil water potential and basal area explained air temperature and soil temperature (Lebrija-Trejos *et al.* 2010).

Air temperature, air relative humidity and soil water contents are not related to any of the stand structure variables studied. One of the factors responsible for soil water content is the accumulation of organic material on the forest floor (Leuschner & Rode 1999), suggesting that the chemical and structural characteristics of the leaves in the canopy play an important role; not only the quantity (related to biomass / stand structure) but also the quality (chemical and structural characteristics) of the litter. As litter production during tropical forest succession increases due to accumulated standing biomass, litter decomposition rates decrease due to the 'fast' high quality leaves (high nutrient contents, high SLA) being replaced by 'slow' species with a lower decomposability (high LDMC, high leaf toughness) (Garnier *et al.* 2004; see also Chapter 2). This predicts an increase of soil organic matter with fallow age (*e.g.* Brown & Lugo 1990, Lawrence & Foster 2002) which is likely to cause an increase in soil water content. This illustrates that leaf traits (quality) are as likely to influence subcanopy environmental conditions as stand structure characteristics (quantity).

Our sites differed in the community aggregated trait-values, both when aggregation was based on trait presence and on trait abundance (Figure 6). The fact that the two PCA-graphs show completely different segregation of sites indicates that trait presence and trait abundance are different aspects of functional ecology. Ecological filters shaping the community, could be described as different (hierarchical) layers of assembly rules (Keddy 1992); first determining presence/ absence and secondly shaping abundance of species with certain traits. Results of this study show that indeed for a given community the functional characteristics of the species that are present (simple community average) are different from the functional characteristics of species that are abundant (community weighted mean), suggesting the presence of distinct multilayered filtering processes. This is substantiated by the finding that the distinct filtering processes are found to be related to different environmental conditions; trait presence is determined by light availability whereas trait dominance is shaped by air temperature and soil water content. This is in line with Cingolani *et al.* (2007) where filters determining the probability of species presence are not necessarily the same as filters determining which species become abundant. They found that functional traits of abundant species (community weighted mean) are explained by moisture and grazing management of grasslands in Argentina whereas simple averages did not always respond to disturbance or abiotic conditions.

The distinct PCA scatterplots when species are weighted for abundances compared to based on presence/absence suggest that dominant species are functionally distinct from the overall range of traits present in sites. In the study design we have particularly tried to

include species with a large range in abundances (dominant as well as minor species). However not all species could be sampled and the missing species (and traits) are biased towards rare species. Therefore, traits represented in the species presence/ absence PCA is not an exhaustive representation of the trait values that are *really* present, but are somewhat biased towards the species that are dominating the sites. This consideration makes the distinctness of the segregation of sites between the two methods (simple versus weighted) even more apparent.

Thus, in our system, species become dominant when they are functionally adapted to air temperature and soil water availability (Figure 8). This suggests that the risk of overheating of the leaves and desiccation due to scarce water availability may be important filters determining plant successfulness in young secondary forests.

For trait-presence, the proportion of light penetrating the canopy plays a major role. This can be explained by the shade-tolerant species entering the secondary forest in the first few years of succession, adapted to persist under different levels of shade, and not becoming dominant until the dominant pioneer species will disappear (Finegan 1996). Indeed, light is a limiting factor for species composition in wet tropical forests (*e.g.* Poorter 1999). Especially the relation between seedling performance and light availability is extensively studied (*e.g.* Kobe 1999; Montgomery & Chazdon 2002). Earlier results of Nicotra *et al.* (1999), show similarities in patterns of light and seedling distribution in Costa Rica, but relations were not strong which they explain by a time-lag between measuring light and seedling appearance. Even though our results show that light is highly significant factor explaining species presence, the time-lag is also important in this study, especially since here we looked at saplings which may have appeared in the selected category (DBH>1cm) years ago.

Caution should be taken when attributing specific environmental conditions to different filters in community assembly. Although the results clearly find specific environmental conditions related to different aspects of trait composition, environmental conditions are not independent. Furthermore, the explanatory conditions discussed above are based on environmental measurements during the dry season, on the hottest and driest time of the day, whether results are the same when environmental conditions are tested outside the dry season remains to be tested although some conditions (especially soil water content) will probably not differ much between sites in the wet season. In Mexican dry forest air temperature was also shown to have the most important filtering effect on (weighted) trait variation among sites (Lebrija-Trejos *et al.* 2010), suggesting that air temperature may deserve more attention in studies on community assembly across different forest types.

During secondary succession wet tropical forests show directional changes in stand structure, altering the understory environment which in turn drives community assembly through the process of filtering of functional traits.

In this study we show that early successional communities (3.5-22.5 years of fallow age) in wet tropical forests are shaped by habitat filtering that selects species traits at different levels. Trait presence is predominantly explained by the amount of light penetrating to the understory. Important filters determining trait successfulness are air temperature and soil water content, suggesting the risk of overheating of the leaves and desiccation due to limited soil water availability to be prominent limitations during the dry season in wet tropical forests.

Grime's mass ratio hypothesis (Grime 1998) states that ecosystem functioning is essentially determined by the trait values of the dominant contributing species. Recognizing the environment (mainly air temperature) as an important factor determining the dominance of species with certain traits, suggests that changes in climate can have serious implications for ecosystem functioning, as previously indicated by Wright & Muller-Landau (*unpublished results*). This finding underlines the potential danger of climate change for the worlds tropical forests and elaborates the need for more research on the effects of climate change on ecosystem functioning.

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# Chapter 2: Functional traits related to changing environmental conditions during secondary succession: Environmental filtering and the slow-fast continuum

## Abstract
Secondary succession is ongoing community assembly. Successional theory predicts that during succession the dominating species change from fast species (rapid acquisition of resources and fast growth) to slow species (conservation of internal resources and long life but slow growth). This change is predicted to be the result of environmental filtering, one of the major community assembly processes. Here we test these predictions. We analyzed understory environmental conditions in a wet forest successional chronosequence (3.5-22.5 years of fallow age), and analyzed selected functional characteristics of their tree communities. We found that the main component separating the 82 species based on the functional traits measured reflected predictions on the slow-fast continuum. As expected, acquisitive/ fast species are characterized by large leaf area, high SLA, large petioles and high quantum yield values. Likewise, conservative/ slow species are characterized by high LDMD, strong leaves, high instantaneous chlorophyll fluorescence and high wood density. During secondary succession the understory environmental conditions changed from high temperatures, high light availability and low relative humidity and soil water contents in young stages to reversed values in older ones. Most community weighted functional traits (5 out of 9) were significantly explained by environmental conditions, substantiating the environmental filtering hypothesis. However, changes in community weighted mean trait values did not always reflect an increased importance of 'slow' traits with environmental conditions typical for older sites. Maybe other not-explicitly-tested functional traits like drought resistance or defense against herbivory are more important community traits.

Keywords: Environmental filtering; Functional traits; Slow-fast continuum; Chronosequence, Wet tropical forest; Secondary succession; Secondary forests, Chiapas, Mexico.

## Introduction
Secondary succession is ongoing community assembly. If environmental filters shape community assembly, particular combinations of traits and environment should be found, in spite of heterogenizing effects of random dispersal or competition that also influence assembly (Hubbell 2001). Studies on changing species composition during secondary succession in tropical rain forests often focus on a gradient of decreasing understory light availability (*e.g.* Bazzaz & Picket 1980) where a trade-off in plant design allows fast growing types with a rapid acquisition of resources under higher light conditions and slow growing types that permit conservation of resources within the plant dominating late successional stages where light becomes an increasingly limited (*e.g.* Poorter 1999; Reich *et al.* 2003). This 'slow-fast continuum', also referred to as the growth-survival trade-off (*e.g.* Kitajima 1994; Sterck *et al.* 2006; Kitajima & Poorter 2008), is shown to be maintained across different geographic regions and is consistent across floras and phylogenetic groups (Díaz *et al.* 2004).

Several species traits may be used to indicate the slow-fast continuum. In this study we use nine traits, covering both morphological and physiological traits reflecting a range of

functional roles in the ecosystem such as light acquisition, relative growth rate, construction costs, photosynthetic performance, conservation of internal resources and vulnerability to damage (Table 1).

Acquisitive or 'fast' species gamble for a quick return of leaf investment by investing in cheap short-lived leaves with high assimilation rates which enable them to grow quickly and outcompete neighbours, thereby remaining at the top of the growing vegetation (Poorter & Bongers 2006). These 'cheap' leaves are characterized by large areas (high leaf area) to increase light interception (Bazzaz & Picket 1980) and a high SLA for increased photosynthetic rates under limited investment (Reich *et al.* 1994; Poorter *et al.* 2009). Fast species will invest in long petioles in order to maximize lightcapture by reducing leaf aggregation around the stem (Takenaka 1994).

Conservative species on the other hand will conserve their resources by investing in persistent leaves: leaves are stronger to better resist herbivory and endure physical damage, with high leaf dry matter content (LDMC) ensuring a long leaf life span and increased persistence (Poorter & Bongers 2006). Also higher thickness of the lamina of the leaves is suggested to reflect increased leaf persistence and lifespan (Reich *et al.* 1991). Wood density is another trait reflecting the trade-off between fast growth and a more conservative strategy. Low wood density species are enabled to grow faster because the soft wood (large vessel diameters) does not cause as much hydraulic resistance, and hence stimulate photosynthetic rates. However, ultimate tree height is limited due to increased risk of xylem cavitation. Late successional species have a higher wood density and are less vulnerable to stem rot and pathogens (Augspurger & Kelly 1984) and eventually a higher maximum height can be reached (Sterck & Bongers 2001). Overall, higher wood density is shown to be correlated to lower growth and mortality rates (Poorter *et al.* 2008; Chave *et al.* 2009) and is in line with species' conservative or slow strategy.

Finally, two physiological traits related to primary productivity are included in the study; instantaneous chlorophyll fluorescence and quantum yield. Fluorescence measurements are based on the knowledge that when chlorophyll takes up a photon to reach the excited state, the available energy will be disposed in one out of three ways: energy is used in photochemistry, it is converted into heat, or emitted in the form of fluorescence (Lambers *et al.* 2008). Quantum yield is the proportion of the light that is *not* emitted in the form of fluorescence but instead used in photochemistry or emitted in the form of heat. We predicted that early successional species have a higher quantum yield, reflecting higher photosynthetic capacity (allocation to photochemistry), as well as a better tolerance against photoinhibition (emission of excess energy in the form of heat is more efficient than in the form of fluorescence (Maxwell & Johnson 2000)). Instantaneous chlorophyll fluorescence is the maximum fluorescence dissipated after a pulse of saturating light and *not* used in photochemistry or emitted as heat, this is expected to reflect late successional strategies as the importance of releasing excess energy most efficiently reduces as light will become limited and the need for high photosynthetic capacity is lower for slow growing species.

The trade-off in functional traits is tested by exploring multivariate trait associations in a principal component analysis.

The current study includes a range of understory environmental conditions (air temperature, air relative humidity, light availability, soil temperature and soil water content) that are expected to reflect changing environmental conditions along a secondary succession gradient. As basal area increases with fallow age, the leaf area index will also increase (*e.g.* Kendal Snell & Brown 1978; Burton *et al.* 1991) which reduces light available in the

understory (Beer-Lambert law). With less light penetrating the canopy, the understory conditions are increasingly characterized by lower temperatures in the air and soil and reduced evaporation causing an increase in the relative humidity and soil water contents. The environmental conditions for each of the secondary forest sites are tested for correlations with the community weighted trait mean trait values per site to test the environmental filtering hypothesis.

This study has the objective to analyze the slow-fast trade-off based on functional traits of species as well as the environmental filtering hypothesis in secondary forests of Chiapas in Mexico. For this first the trade-off in functional traits is tested by separating species in a principal component analysis based on species trait averages and analyzing the multivariate trait associations. Secondly we tested the environmental filtering hypothesis by checking for correlation between traits at the plot level (Community Weighted Mean) and environmental conditions along a gradient of succession. We hypothesize that in time since abandonment the community weighted mean (CWM) in leaf area (LA) will decrease, specific leaf area (SLA) will decrease, leaf dry matter content (LDMC) will increase, leaf thickness (LT) will increase, leaf strength (sFtP) will increase, petiole length (PL) will decrease, instantaneous chlorophyll fluorescence ($F_o$) will increase, quantum yield (QY) will decrease and wood density (WD) will increase. Secondary succession will reflect a gradient in environmental conditions from high temperature, low relative humidity, low soil water content and high light availability in young secondary forests to reversed values in older sites. The changes in community weighted mean will reflect the changes in species composition from species with acquisitive 'fast' characteristics to species with conservative 'slow' characteristics.

*Table 1. Leaf trait abbreviations, a short description of their functional role, references used and their hypothesized relation to time after abandonment.*

| Trait; abbreviation (units) | Functional role | References | Expectation along succession |
|---|---|---|---|
| Leaf area; LA ($cm^2$) | Light intercepting area, dry matter production, respiration, leaf cooling, gas exchange | Bazzaz & Picket 1980 Popma *et al.* 1992 | decrease |
| Specific Leaf Area; SLA ($m^2 Kg^{-1}$) | Light capture economics, net assimilation rate, relative growth rate, leaf life span, photosynthetic capacity | Reich *et al.* 1997 Poorter & Garnier 1999 Sterck *et al.* 2006 Poorter & Bongers 2006 | decrease |
| Leaf Dry Matter Content; LDMC ($g\,g^{-1}$) | Leaf tissue density, indicator of assimilative compounds and transfer conductance to $CO_2$, construction costs and nutrient retention, against mechanical and herbivore damage, tolerance against water limitations | Poorter & Garnier 1999 Niinemets 2001 Garnier *et al.* 2004 | increase |
| Leaf Thickness; LT (mm) | Construction costs, leaf life span, photosynthetic rates per unit leaf area, against | Reich *et al.* 1991 Popma *et al.* 1992 Niinemets 2001 | increase |

| | mechanical and herbivore damage, gas exchange and leaf cooling | Díaz *et al.* 2004 Onoda *et al.* submitted | |
|---|---|---|---|
| Specific Force to Punch; sFtP (MN $m^{-2}$) | Leaf strength, survival of herbivory and physical damage, persistence, leaf lifespan | Coley 1983 Kitajima & Poorter 2010 Onoda *et al.* submitted | increase |
| Petiole length; PL (cm) | Light capture efficiency | Black 1960 Takenaka 1994 | decrease |
| Instantaneous chlorophyll fluorescence; $F_0$ (no unit) | Disability to dissipate heat, resistance against overheating, respiration, photosynthetic performance | Maxwell & Johnson 2000 Baker & Rosenqvist 2004 | increase |
| Quantum Yield; QY (no unit) | Effectiveness of photosystem II, $CO_2$ assimilation, stress tolerance, photosynthetic performance, leaf nutrient levels | Maxwell & Johnson 2000 Parkhill *et al.* 2001 Baker & Rosenqvist 2004 Dos Santos *et al.* 2006 | decrease |
| Wood Density; WD (g $cm^{-3}$) | Construction costs, growth rate, stem vulnerability, mortality rate | Augspurger & Kelly 1984 van Gelder *et al.* 2006 Poorter *et al.* 2008 Chave *et al.* 2009 | increase |

**Methods**

*Study site*

The research location for this study is near the Chajul tropical field station, south of the Montes Azules Integral Biosphere Reserve, at the border of the Marques de Comillas region, within the Lacandon region in southeast Mexico (16°01'N, 90°55'W). The climate is warm-humid with an average annual temperature of 25-27°C and an annual rainfall of 3.000 mm, with less than 100 mm per month falling in the dry season (February-April) (Ibarra-Manríquez & Martínez-Ramos 2002; Breugel *et al.* 2006). The study takes place around the village of Loma Bonita, an area characterized by small hills and valleys with sandy or limestone soils of low pH (<5.5). Currently 65% of the land area around Loma Bonita is dedicated to agricultural practices (mostly livestock pasture), 14% is mature forest and 21% consist of secondary forests of different ages. The old growth forests around Loma Bonita have suffered extensive pressure for changing land-uses, even more so than surrounding villages, possibly due to colonization of refugees from Guatemala since '82, giving rise to the current large area of secondary forests (Zermeño-Hernández 2008).

The fallow age of the secondary forest sites is determined based on information of landowners and other local residents (Breugel *et al.* 2007). The chronosequence consists of 11 sites (10 x 50 m) with fallow ages between 3.5 and 22.5 years (plot 1-11 reflect increasing fallow age). All sites are established on abandoned corn fields, the main form of agriculture, and plots were in use within one year before abandonment.

*Environmental variables*

To test for consistent trait-environment relations across the secondary forest sites, five environmental variables have been measured: air temperature, air relative humidity, light availability, soil temperature and soil water content.

The sub-canopy environmental variables were measured during the dry season (March and April). We measured air temperature and relative humidity in the vegetation (height of 1.5m) by using HOBO temperature / relative humidity data loggers (HOBO Pro v2 Temp/RH logger, Onset Computer Corporation, UK). For each of the 10 HOBO dataloggers a measurement was taken every minute for 15 minutes. The percentage of canopy openness (here measured as an indicator of light availability) is measured with a spherical densiometer (Spherical Densiometer Concave). A densiometer is sometimes criticized for not being very accurate in measuring light conditions (*e.g.* Engelbrecht & Herz 2001), but sites showed very distinct levels of light availability (ranging between 15% to 70% of openness or light availability) and the relative simplicity of the canopy in secondary sites compared to old growth forest (*e.g.* Brown & Lugo 1990) legitimizes the use of this method. Also two independent days of sampling have been used to compromise for accuracy, all measurements taken by the same person. Soil temperature (°C) and water content (% volume) were assessed with a W.E.T. Sensor (type WET-2, Delta-T Devices Ltd) at a depth of about 10 cm.

The environmental variables have been measured for 3 days (2 days in case of densiometer measurements) during the dry season, at least 3 days after the last rain, on days without overcast. Dates measuring subcanopy environmental conditions are randomly spread over the sampling period. Measurements were carried out at the hottest and driest part of the day, between 13.00 and 16.00. This is expected to capture the most extreme conditions to which plants need to be adapted and considered a good way to illustrate differences between the sites. Per plot 10 replicates were taken at even distances in a transect of 50 meters at the center of the plot. (Site 3 only allowed 4 replicate measures instead of 10 because it had partly been cleared.)

*Functional traits*

Nine functionally important traits were studied on saplings up to 5 meters high. Leaf traits have been measured on 2 (sun-lit) leaves per individual and 10 replicate individuals per species, except for specific force to punch where 5 individuals per species were assessed. Wood traits are based on five adult individuals per species.

Standardized measurement protocols have been carried out as suggested by Cornelissen *et al.* (2003).

*Leaf Area* (cm$^2$). Leaf area was calculated by photographing the fresh area excluding the petiole on a lightbox after which the area was calculated with pixel counting software ImageJ (Rasband 2008).

*Specific Leaf Area* (m$^2$ Kg$^{-1}$). SLA was calculated as the fresh leaf area divided by the oven dried mass (dried for 48 hours at 70°C), excluding the petiole. In the case of composite leaves the smallest photosynthetic unit was taken.

*Leaf Dry Matter Content* (g g$^{-1}$). After collection the leaves were placed in water to rehydrate for at least 5 hours after which the fresh weight has been determined. The oven dry weight is divided over the fresh weight for acquiring the leaf dry matter content.

*Leaf thickness* (mm). Determined with a digital caliper in the middle of the leaf avoiding the main and secondary nerves.

*Specific Force to Punch* (MN m$^{-2}$). The laminas of fresh leaves (center of the leaf, avoiding primary and secondary nerves) were perforated with a penetrometer built with a flat-end nail attached to the inner part of a syringe and a waterbasin on top. Water was added to the basin until the leaf ruptures. The total weight added was converted into Mega Newton and divided over the breaking surface (circumference of the nail x thickness of the leaf). This alternative punching method shows similar trends compared to cutting and tearing tests and is a good indicator of leaf mechanical resistance (Onoda *et al. submitted*).

*Petiole length* (cm). Determined with a digital caliper or a tape-measure.

*Instantaneous Chlorophyll Fluorescence ($F_0$)*. The fluorescence on exposal to light (saturating light 455 nm at an intensity of 3000uE) after dark adaptation (1 hour) was measured with a fluorpen (Photon Systems Instruments, FP100).

*Quantum Yield ($F_v / F_m$)*. The ratio between the variable fluorescence and maximum fluorescence on exposure to light (saturating light 455 nm at an intensity of 3000uE) after dark adaptation (1 hour), this reflects the proportion of light used in photochemistry or emitted as heat. Measurements are taken with a fluorpen (Photon Systems Instruments, FP100).

*Wood Density* (g cm$^{-3}$). With an increment borer (12" mm Suunto, Finland) a core of wood was extracted up to at least the heart of the tree (firmly attached bark or phloem tissue was considered part of the stem). For some species the stems did not reach sufficient size for this method and alternatively a slice of the stem was extracted. The wood cores were divided in three parts (across the heart, inner wood and outer wood) and the wood density is measured for these parts separately by dividing the dry weight over the fresh volume (water replacement method). These three parts are averaged for the species wood density. This measurement was taken for 32 of the 82 species studied, data on wood density for remaining species were taken from comparable studies in Mexican wet forests in Chajul (Chiapas), Las Margeritas (Puebla) and Los Tuxlas (Veracruz) (Martínez-Ramos & Bongers *unpublished results*).

The traits are scaled up to the community level by the Community Weighted Mean (CWM) (Díaz *et al.* 2007; Lavorel *et al.* 2007), a parameter that can be seen as the functional value of a randomly sampled plant from the community.

It is calculated as:     $$CWM = \sum_{i=1}^{s} W_i\, x_i$$

Where S is the total number of species, $W_i$ is the relative importance of the ith species and is the trait value of the ith species. The importance value reflects a species proportion of all saplings at the site (DBH > 1cm; between 1.3 and 3 m high) based on the relative number of stems and the relative basal area. It is calculated as: $(BA_i/BA_{total} + D_i/D_{total})/ 2$. Where $BA_i$ is the basal area of the ith species, $D_i$ is the density of the ith species and total reflects the total of individuals in the selected sapling category.

*Statistical analysis*

To test for the trade-off in plant strategies among species a PCA ordination was carried out. The multivariate trait associations among the 82 species are based on simple species averages per trait and are analyzed for consistency with the slow-fast continuum. A cluster

analysis was carried out (Euclidean distance, average linkage between groups) to identify whether the secondary forest species could be clustered into functional groups. Another PCA was performed to separate sites based on the understory environmental conditions, the PCA axes were subsequently related to time since abandonment with curve estimation analysis.

A multiple linear regression (backwards) is used to assess consistent trait-environment linkages to test the environmental filtering hypothesis. For this the plot-level trait value was calculated by the community weighted mean. SPSS 17.0 was used, where data was not normally distributed, transformations were carried out to improve parametric model fit.

**Results**

The 82 selected secondary forest species in the Chajul area exhibited a large variation in functional strategies (Appendix 1; Figure 1). The first axis of the PCA based on species trait averages explained 32% of total variation and is shaped by increasing LA, increasing SLA, decreasing LDMC, decreasing sFtP, increasing PL, decreasing $F_0$, increasing QY and decreasing WD . The second axis (explaining 21%) is determined by increasing LA, SLA, LDMC, decreasing LT, increasing sFtP, PL, $F_0$, Qy and WD.
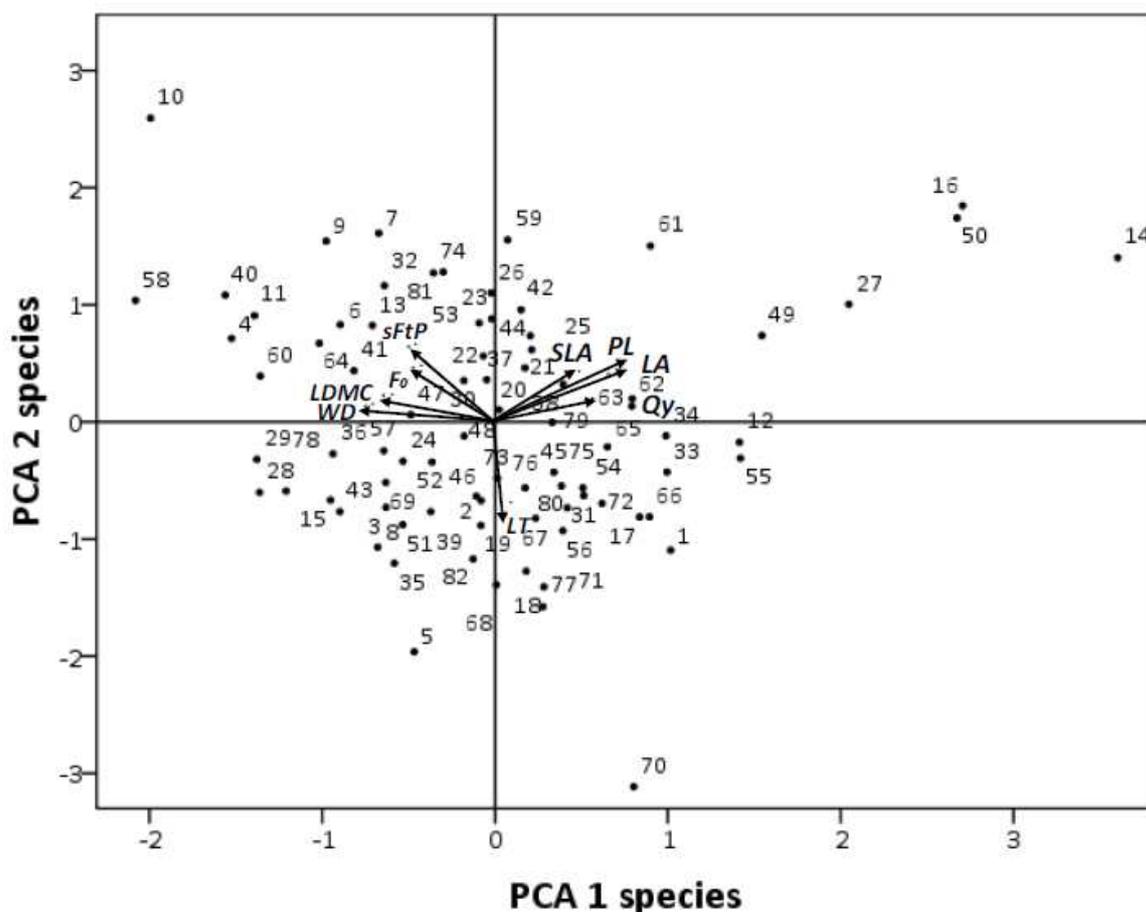


*Figure 1. PCA ordination of Chajul's 82 species included in this study based on their functional traits. With increasing value of PCA axis 1 species are increasingly characterized by fast (acquisitive) traits such as high leaf area (LA) and SLA, low leaf dry matter content (LDMC), low force to punch (sFtP), large petioles (PL), low instantaneous chlorophyll*

*fluorescence ($F_0$), high quantum yield (QY) and a low wood density (WD). The numbers indicate the following species: 1= Aegiphila monstrosa, 2= Alchornea latifolia, 3= Alibertia edulis, 4= Ampelocera hottlei, 5= Amphitecna apiculata, 6= Arageborita sp., 7= Astronium graveolens, 8= Bellucia axinanthera, 9= Brosimum alicastrum, 10= Brosimum costaricanum, 11= Brosimum guianensis, 12= Bursera simaruba, 13= Calophyllum brasiliense, 14= Carica papaya, 15= Casearia sylvestris, 16= Cecropia peltata, 17= Ceiba pentandra, 18= Conostegia xalapensis, 19= Cordia alliodora, 20= Cordia diversifolia, 21= Croton schiedeanus, 22= Cupania dentata, 23= Cupania glabra, 24= Dalbergia glabra, 25= Dendropanax arboreus, 26= Dialium guianense, 27= Erythrina folkersii, 28= Eugenia acapulcensis, 29= Eugenia nigrita, 30= Faramea occidentalis, 31= Gliricidia sepium, 32= Guarea glabra, 33= Hamelia patens, 34= Heliocarpus appendiculatus, 35= Hirtella americana, 36= Hirtella racemosa, 37= Inga pavoniana, 38= Iresine arbuscula, 39= Lacistema aggregatum, 40= Licania hypoleuca, 41= Licania platypus, 42= Lonchocarpus castilloi, 43= Luehea speciosa, 44= Miconia ampia, 45= Miconia glaberrima, 46= Miconia trinervia, 47= Nectandra reticulata, 48= Nectandra salicifolia, 49= Ochroma piramidale, 50= Oreopanax peltatus, 51= Parathesis lenticellata, 52= Phylostilum subsecile, 53= Piper aduncum, 54= Piper aff. hispidum, 55= Piper auritum, 56= Piper sanguinea, 57= Posoqueria latifolia, 58= Pouteria durlandii, 59= Protium copal, 60= Psidium friedrichsthalianum, 61= Pterocarpus rohrii, 62= Schizolobium parahybum, 63= Senna papillosa, 64= Sideroxylon nigra, 65= Siparuna andina, 66= Solanum rudepanum, 67= Solanum umbelatum, 68= Tabernamontana alba, 69= Terminalia amazonia, 70= Thevetia ahouai, 71= Trema laxiflora, 72= Trema micrantha, 73= Trichospermum mexicanum, 74= Vatairea lundellii, 75= Vernonia patens, 76= Vismia camparaguey, 77= Vochysia guatemalensis, 78= Xylopia frutescens, 79= Zanthoxylum caribaeum, 80= Zanthoxylum kellermanii, 81= Zanthoxylum procerum, 82= Zuelania guidonia.*

A species clustering based on species functional traits (squared Euclidean distance, average linkage between groups), resulted in 2 groups where *Carica papaya* (14), *Cecropia peltata* (16) and *Oreopanax peltatus* (50) form one group and are separated from the rest. A separate analysis on the main species group (79 species) showed that the first principal component still reflects the trait associations found but now explains 29.9% of the variance between species (results not shown).

The sites studied vary in sub-canopy abiotic conditions. 82% of the variation among plots is explained by the first two principal components axes (58% and 24% respectively), reflecting differences in air temperature, air relative humidity, light availability, soil temperature and soil water content (Figure 2). The first axis of the environmental PCA correlated significantly with time since abandonment in an inverse model ($R^2$= .653; p= .003) (Figure 3). Young sites are characterized by high light availability and high temperatures of the soil and air whereas older secondary sites are characterized by high relative humidity and soil water contents.
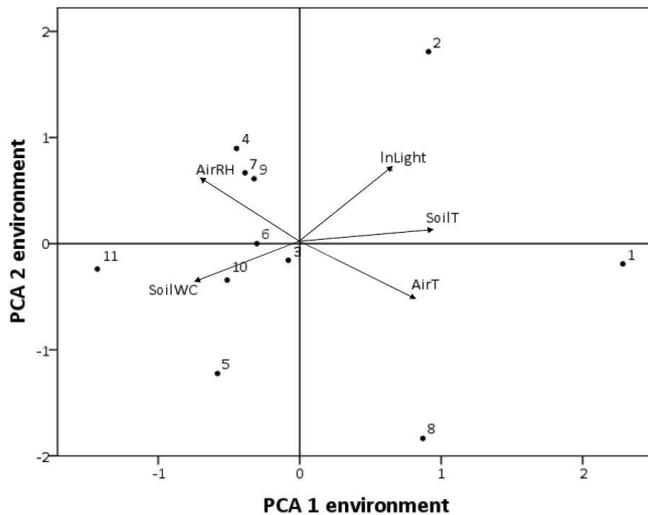
*Figure 2. The secondary forest sites separated based on the subcanopy environmental conditions (air relative humidity, air temperature, light availability, soil water content, soil temperature). The first axis explains 58% and the second axis 24% of the environmental variation between the sites.*
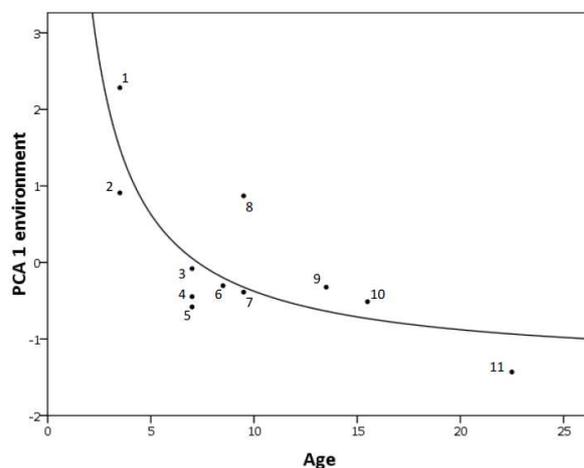


*Figure 3. The first component of the PCA (58%) separating sites based on their subcanopy environmental conditions is significantly explained by age of the secondary forest ($R^2$= .653; p= .003).*

Community weighted mean trait values (CWM) per plot are well explained by the environmental variables. Leaf area is explained by variation in air temperature (R= .614; p= .044), leaf thickness is related to light availability (R= -.578; p= .063), leaf strength is related to a combination of soil temperature (R= -.797; p= .065), air relative humidity (R= .825; p= .046) and air temperature (R= 1.556; p= .012). Petiole length is explained by air temperature (R= .600; p= .051) and quantum yield is related to light availability (R= .652; p= .019) and soil water contents (R= 1.020; p= .002) (Figure 4). Surprisingly, several CWM trait values (SLA, LDMC, $F_o$ and WD) are independent of the environmental variables measured.
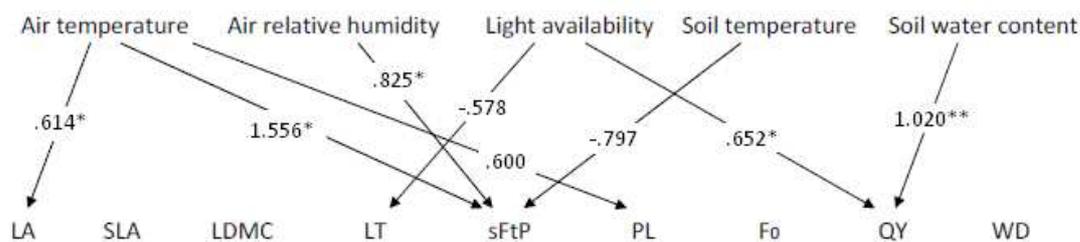
*Figure 4. Trait-environment relations showing the environmental variables (air temperature, air relative humidity, light availability, soil temperature and soil water content) that significantly explain variation in the community weighted mean (CWM) trait values (Leaf Area, Specific Leaf area, Leaf Dry Matter Content, Leaf Thickness, specific Force to Punch, Petiole Length, Instantaneous chlorophyll fluorescence, Quantum Yield, Wood Density) as selected by the multiple linear regression (\* p < .05, \*\* p < .001).*

**Discussion**

The 82 species included in this study show a large variation in functional strategies. Most of the trait variation between species is in line with the hypothesized fast-slow continuum. Indeed the 'slow' or conservative species are characterized by a high force to punch (sFtP), high instantaneous chlorophyll fluorescence ($F_0$), high leaf dry matter content (LDMC), high wood density (WD), low specific leaf area (SLA), low Quantum yield (QY) and short petioles (PL) (Table 1, Figure 1). These results are in line with earlier studies based on global datasets (Wright *et al.* 2004; Díaz *et al.* 2004) and datasets on tropical regions where trait correlations have been attributed to successional strategies (Poorter *et al.* 2006;  Sterck *et al.* 2006). Clustering of species into functional groups results in a small group with only *Carica papaya* (14)*, Cecropia peltata* (16) and *Oreopanax peltatus* (50) and a second group with all other species. These three species are indeed typical acquisitive/ pioneer species (Figure 1). The cloud of species in the PCA illustrates that functional strategies cannot easily be grouped since variation in traits among species is continuous rather than categorical (*e.g.* Wright *et al.* 2004; Petchey & Gaston 2006). To check whether the trade-off between rapid acquisition and conservation of resources is consistent across a large range of species and not just pulled by few extremes, we excluded the three species after which another PCA is generated. The first component still reflects the slow-fast continuum indicating that our findings are not just pulled by the three pioneer species *Carica papaya, Cecropia peltata* and *Oreopanax peltatus* but is consistent across a the range of 82 species studied.

Our results indicate that the trade-off between acquisitive species and conservative species is prominent among tropical tree species in wet secondary forests. Fast species achieve high relative growth rate by maximizing light interception (large leaves, large petioles) and maximizing productivity and photosynthesis (high SLA, high quantum yield, low instantaneous chlorophyll fluorescence). On the contrary, 'slow' conservative species invest in strong leaves (high sFtP, high LDMC) and dense wood (high wood density) to endure physical damage and lower mortality. Leaf thickness, a measure of leaf investment and a proxy for leaf lifespan (Reich *et al.* 1991), is largely independent of the fast-slow continuum of strategies in this study unlike earlier findings of Diaz *et al.* (2004) in a global dataset. However Kitajima & Poorter (2010) show that leaf thickness is not a good proxy for leaf lifespan when only tropical forest species are assessed, substantiating that leaf thickness may indeed be irrelevant to the slow-fast continuum in tropical forests.

It should be noted that this PCA is based on traits that are selected for their known relation to the trade-off in resource acquisition strategies, when traits like seed size and maximum height (more related to the colonization- competition trade-off) would have been included the spectrum may have been different.

The 11 secondary forest sites show a gradient of understory environmental conditions that is significantly related to the age of the site. Young secondary sites are characterized by high temperatures of air and soil, high light environment and low relative humidity and soil water contents (Figure 3). The inverse model illustrates that mainly the very young sites (<5 years) exhibit the extreme hot and dry conditions. Already at about 10 years of fallow age the environmental conditions seem to have reached a relatively constant environment considering the variables studied. Site 8 (9.5 years of fallow age) is characterized by an environment more typical for the very young successional sites and appears as an outlier in the graph. This may be due to it's location which is somewhat higher in elevation than the other sites, possibly causing it to be drier.

The community weighted mean for five out of nine functional traits are significantly related to the abiotic conditions (Figure 4). This is a strong indicator that environmental filtering is an important  driver of community assembly in the study area.

Leaf area is significantly larger in sites with higher temperatures, confirming the hypothesis that early successional species maximize light capture for fast growth (Bazzaz & Picket 1980). Leaves are thicker in sites with less light available in the understory, also in line with expectations. Older plots with complex multilayered canopies allow less light to penetrate, and species adapted to the low light conditions of older secondary forests will be characterized by conservation of internal resources like thick and durable leaves (Onoda *et al.* submitted).

Leaf strength is explained by a combination of the variables air temperature, air relative humidity and soil temperature, in which air temperature explains the main variation. Results indicate that leaves are physically stronger in the young sites with higher temperatures, rejecting our hypotheses. Accordingly, leaves in older sites appear thicker, but not stronger as leading from the trait-environment relations. In this study leaf thickness and specific force to punch are not related (Appendix 2) unlike previous studies demonstrated (Santiago 2003; Onoda *et al. submitted*). This can be explained by our measure of leaf strength which is normalized for breaking surface, and thus also for lamina thickness, ignoring the contribution of thickness to leaf strength (Read & Sanson 2003). Therefore this suggests that the importance of *absolute* leaf strength may increase during successional changes, like hypothesized, whereas the specific force to punch (leaf strength per unit breaking surface) does not. An earlier study on leaf toughness in secondary forests on highly degraded versus fertile soils showed that leaf toughness was higher on degraded soils (Choong *et al.* 1992), probably because  plants on fertile soils do not need to defend their leaves, as they can easily replace leaf loss by herbivores (resource availability hypothesis). This provides another explanation for stronger leaves in younger sites: these sites are more degraded. A second alternative hypothesis for increased leaf strength in high temperature sites may be found in the leaf hydraulic capacity, defined as a plant's capacity for water use and is negatively correlated to gas exchange (Sack & Frole 2006). We expect a lower hydraulic resistance in leaves of pioneer species because of increased need for gas exchange to attain high levels of photosynthesis. This may be achieved by more venation which is likely to increase leaf

strength since toughness outside the primary and secondary nerves is mainly determined by the tertiary and higher order veins of the leaf (Choong *et al.* 1992).

The length of the petiole shows directional changes and is positively correlated to air temperature, as hypothesized. Early successional species in the high light environments maximize light capture by selection for large petioles that reduce leaf aggregation around the stem (Takenaka 1994). This result strengthens the positive relation between leaf area and air temperature because leaf size and petiole length are allometrically related (Niinemets & Kull 1999), as also shown in this study (Appendix 2).

Quantum yield is explained by a combination of the light availability and soil water contents where soil water contents explains the main variation. Against hypothesized, the positive relation between quantum yield and water contents of the soil indicates that plants in late successional plots have a more efficient strategy to use excess light in photosynthesis (Dos Santos 2006) and more resistance against stress which can cause damage to the photosystem II (Maxwell & Johnson 2000; Parkhill *et al.* 2001). However, quantum yield is an indication of the energy used in photochemistry *or* emitted as heat without knowing the proportion attributed to both processes. One could reason that the photosystem II efficiency needs to be higher for late successional species, as less sunlight is available which would increase the need for efficient use of energy in photochemistry. A higher resistance against photoinhibition by the ability to emit energy in the form of heat (instead of in the form of fluorescence) does appear relevant for late successional species and maybe in this case the higher quantum yield should only be attributed to the efficiency of energy use in photochemistry.

Specific leaf area, leaf dry matter content, instantaneous chlorophyll fluorescence and wood density show no pattern with any of the environmental variables measured. Lack of pattern of instantaneous chlorophyll fluorescence is possibly due to high plasticity hampering the comparison between species (Maxwell & Johnson 2000) which could also be the case for SLA and LDMC. Another reason for the lack of response of sapling traits to subcanopy environmental conditions is that traits can be subjected to opposing gradients. Besides the fast-to-slow gradient along succession there may be selection for extreme stress tolerant species in the very early hot, dry and open sites. For example for leaf dry matter content alternative predictions have been formulated such as decreasing LDMC during succession as a reflection of tolerance against water limitations (Niinemets 2001). Plant adaptations to do with defense against herbivory or pathogens were also proposed to explain results that were contrary to hypothesized from the slow-fast trade-off in Ricaño-Rocha (2007) based on the lack of pattern in the root-shoot ratio of tropical forest seedlings in the same study area. Chave *et al.* (2009) state that wood properties are not inherently related with 'slow' and 'fast' strategies as found in leaves, concluded from mixed findings correlating wood traits with leaf traits.

Environmental filtering as a driver of community assembly assumes that plants present have survived certain abiotic conditions based on the suitability of their functional traits. However, as environmental conditions are changing during secondary succession, at the actual time of filtering the conditions may have been different from what has been measured at the sites, making the causal relation between traits and their environment arbitrary. A time lag may well be a reason why some traits did not respond, like wood density which is a 'whole plant trait' and thus slower in responses to local abiotic conditions than leaf traits which more easily react to changing abiotic conditions.

**Conclusion**

Data show that the trade-off between species functional strategies for either acquisitive use of resources or conservative use of resources, the slow-fast continuum, explains most of the trait variation in tree species in tropical secondary forest in Chiapas, Mexico. Linking plant functional strategies to changing environmental conditions along a gradient of succession resulted in some strong trait-environment relations, supporting the environmental filtering hypothesis. However not all relations can be explained by the slow-fast continuum and plant adaptations to opposing gradients should be thought of. Besides the growth-survival trade-off, fast versus slow species, adaptation to opposing gradients like exposure to herbivory and pathogens or stress-tolerance may increase our understanding of the wide range of functional strategies present in tropical wet forest.

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**Appendix 2**

Species mean trait values for the functional traits leaf area (LA; cm$^2$), specific leaf area (SLA; m$^2$ Kg$^{-1}$), leaf dry matter content (LDMC; g g$^{-1}$), leaf thickness (LT; mm), specific force to punch (sFtP; MN m$^{-2}$), petiole length (PL; cm), Instantaneous chlorophyll fluorescence (F$_0$; *no unit*), quantum yield (QY; *no unit*), wood density (WD; g cm$^{-3}$).

| | Species | LA | SLA | LDMC | LT | sFtP | PL | F$_0$ | Qy | WD |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Aegiphila monstrosa | 272 | 14.1 | 0.24 | 0.22 | 0.79 | 5.78 | 180 | 0.75 | 0.41 |
| 2 | Alchornea latifolia | 148 | 9.9 | 0.42 | 0.23 | 1.84 | 6.65 | 187 | 0.71 | 0.37 |
| 3 | Alibertia edulis | 43 | 9.4 | 0.47 | 0.21 | 1.23 | 1.06 | 199 | 0.73 | 0.67 |
| 4 | Ampelocera hottlei | 122 | 11.8 | 0.43 | 0.20 | 2.58 | 1.03 | 276 | 0.70 | 0.83 |
| 5 | Amphitecna apiculata | 191 | 8.8 | 0.35 | 0.45 | 1.89 | 0.39 | 253 | 0.70 | 0.42 |
| 6 | Aragebortia sp | 121 | 13.8 | 0.47 | 0.16 | 1.96 | 1.48 | 263 | 0.75 | 0.71 |
| 7 | Astronium graveolens | 119 | 23.8 | 0.48 | 0.08 | 1.89 | 6.99 | 241 | 0.72 | 0.77 |
| 8 | Bellucia axinanthera | 377 | 7.1 | 0.42 | 0.27 | 1.04 | 3.56 | 246 | 0.68 | 0.58 |
| 9 | Brosimum alicastrum | 57 | 18.0 | 0.47 | 0.12 | 2.80 | 0.92 | 284 | 0.72 | 0.44 |
| 10 | Brosimum costaricanum | 105 | 19.8 | 0.41 | 0.09 | 4.21 | 0.96 | 304 | 0.66 | 0.71 |
| 11 | Brosimum guianensis | 31 | 15.9 | 0.43 | 0.13 | 2.04 | 0.79 | 258 | 0.70 | 0.87 |
| 12 | Bursera simaruba | 337 | 25.1 | 0.25 | 0.20 | 1.30 | 9.07 | 179 | 0.74 | 0.24 |
| 13 | Calophyllum brasiliense | 65 | 14.2 | 0.39 | 0.17 | 2.71 | 1.66 | 251 | 0.74 | 0.58 |
| 14 | Carica papaya | 1777 | 17.4 | 0.24 | 0.15 | 1.32 | 57.28 | 155 | 0.78 | 0.11 |
| 15 | Casearia sylvestris | 24 | 10.1 | 0.48 | 0.20 | 1.30 | 0.46 | 213 | 0.68 | 0.59 |
| 16 | Cecropia peltata | 2506 | 14.1 | 0.38 | 0.16 | 1.69 | 46.56 | 174 | 0.76 | 0.30 |
| 17 | Ceiba pentandra | 205 | 13.1 | 0.40 | 0.22 | 1.15 | 14.08 | 169 | 0.72 | 0.19 |
| 18 | Conostegia xalapensis | 51 | 11.3 | 0.34 | 0.25 | 0.62 | 2.94 | 186 | 0.76 | 0.60 |
| 19 | Cordia alliodora | 63 | 11.4 | 0.42 | 0.20 | 1.19 | 2.30 | 179 | 0.72 | 0.46 |
| 20 | Cordia diversifolia | 76 | 18.6 | 0.38 | 0.15 | 1.51 | 2.29 | 207 | 0.72 | 0.45 |
| 21 | Croton schiedeanus | 109 | 20.4 | 0.28 | 0.17 | 1.49 | 2.12 | 270 | 0.75 | 0.48 |
| 22 | Cupania dentata | 815 | 12.1 | 0.43 | 0.18 | 1.97 | 9.36 | 216 | 0.72 | 0.56 |
| 23 | Cupania glabra | 676 | 17.5 | 0.32 | 0.17 | 2.37 | 6.90 | 234 | 0.71 | 0.54 |
| 24 | Dalbergia glabra | 164 | 14.6 | 0.41 | 0.20 | 1.98 | 2.33 | 140 | 0.72 | 0.88 |
| 25 | Dendropanax arboreus | 102 | 19.2 | 0.27 | 0.17 | 2.32 | 6.17 | 247 | 0.75 | 0.46 |
| 26 | Dialium guianense | 91 | 24.0 | 0.36 | 0.13 | 1.92 | 1.54 | 264 | 0.74 | 0.45 |
| 27 | Erythrina folkersii | 589 | 39.5 | 0.18 | 0.18 | 1.63 | 18.59 | 181 | 0.74 | 0.38 |
| 28 | Eugenia acapulcensis | 10 | 9.0 | 0.49 | 0.21 | 1.16 | 0.63 | 249 | 0.67 | 0.73 |
| 29 | Eugenia nigrita | 20 | 8.9 | 0.52 | 0.22 | 1.83 | 0.66 | 229 | 0.70 | 0.72 |
| 30 | Faramea occidentalis | 77 | 16.0 | 0.33 | 0.18 | 1.77 | 1.03 | 259 | 0.75 | 0.55 |
| 31 | Gliricidia sepium | 111 | 20.2 | 0.24 | 0.15 | 0.61 | 3.19 | 157 | 0.74 | 0.79 |
| 32 | Guarea glabra | 78 | 17.3 | 0.37 | 0.13 | 2.32 | 1.28 | 284 | 0.73 | 0.51 |
| 33 | Hamelia patens | 56 | 27.7 | 0.21 | 0.18 | 0.56 | 2.94 | 208 | 0.73 | 0.47 |
| 34 | Heliocarpus appendiculatus | 156 | 19.9 | 0.34 | 0.13 | 0.79 | 8.99 | 192 | 0.74 | 0.26 |
| 35 | Hirtella americana | 56 | 9.2 | 0.45 | 0.28 | 1.26 | 0.40 | 185 | 0.74 | 0.78 |
| 36 | Hirtella racemosa | 10 | 12.0 | 0.49 | 0.17 | 1.69 | 0.24 | 175 | 0.71 | 0.77 |
| 37 | Inga pavoniana | 720 | 18.6 | 0.39 | 0.18 | 1.88 | 3.74 | 219 | 0.67 | 0.42 |
| 38 | Iresine arbuscula | 47 | 21.6 | 0.25 | 0.17 | 1.56 | 1.11 | 249 | 0.76 | 0.48 |

| | Species | LA | SLA | LDMC | LT | sFtP | PL | $F_0$ | Qy | WD |
|---|---|---|---|---|---|---|---|---|---|---|
| 39 | Lacistema aggregatum | 53 | 14.6 | 0.30 | 0.24 | 1.30 | 0.76 | 241 | 0.69 | 0.58 |
| 40 | Licania hypoleuca | 31 | 13.9 | 0.52 | 0.13 | 2.23 | 0.52 | 258 | 0.72 | 0.86 |
| 41 | Licania platypus | 236 | 11.8 | 0.45 | 0.18 | 2.22 | 1.17 | 233 | 0.73 | 0.65 |
| 42 | Lonchocarpus castilloi | 121 | 24.4 | 0.41 | 0.12 | 1.38 | 6.29 | 230 | 0.75 | 0.57 |
| 43 | Luehea speciosa | 151 | 11.2 | 0.55 | 0.20 | 1.40 | 1.23 | 155 | 0.68 | 0.67 |
| 44 | Miconia ampia | 542 | 15.6 | 0.34 | 0.16 | 1.15 | 10.47 | 260 | 0.75 | 0.67 |
| 45 | Miconia glaberrima | 94 | 17.8 | 0.26 | 0.21 | 1.28 | 2.10 | 216 | 0.74 | 0.55 |
| 46 | Miconia trinervia | 220 | 11.3 | 0.34 | 0.19 | 0.88 | 1.98 | 229 | 0.70 | 0.47 |
| 47 | Nectandra reticulata | 36 | 13.3 | 0.44 | 0.18 | 1.62 | 1.25 | 246 | 0.73 | 0.45 |
| 48 | Nectandra salicifolia | 47 | 16.2 | 0.43 | 0.17 | 1.33 | 0.79 | 223 | 0.72 | 0.39 |
| 49 | Ochroma pyramidale | 1066 | 15.6 | 0.40 | 0.14 | 1.37 | 28.43 | 178 | 0.72 | 0.19 |
| 50 | Oreopanax peltatus | 2265 | 15.0 | 0.29 | 0.22 | 1.14 | 61.25 | 257 | 0.72 | 0.37 |
| 51 | Parathesis lenticellata | 110 | 10.9 | 0.30 | 0.22 | 1.34 | 1.58 | 213 | 0.69 | 0.71 |
| 52 | Phylostilum subsecile | 129 | 13.8 | 0.31 | 0.21 | 1.86 | 0.44 | 208 | 0.71 | 0.65 |
| 53 | Piper aduncum | 119 | 19.8 | 0.27 | 0.15 | 2.25 | 0.87 | 283 | 0.72 | 0.33 |
| 54 | Piper aff. hispidum | 99 | 19.1 | 0.26 | 0.19 | 1.02 | 0.54 | 219 | 0.72 | 0.36 |
| 55 | Piper auritum | 609 | 20.6 | 0.21 | 0.19 | 1.16 | 7.63 | 202 | 0.71 | 0.11 |
| 56 | Piper sanguinea | 135 | 16.3 | 0.23 | 0.23 | 0.79 | 0.94 | 230 | 0.72 | 0.48 |
| 57 | Posoqueria latifolia | 114 | 10.6 | 0.32 | 0.23 | 2.18 | 1.43 | 238 | 0.72 | 0.63 |
| 58 | Pouteria durlandii | 156 | 9.9 | 0.46 | 0.16 | 3.30 | 2.65 | 258 | 0.64 | 0.8 |
| 59 | Protium copal | 719 | 17.3 | 0.45 | 0.14 | 2.23 | 12.46 | 248 | 0.75 | 0.52 |
| 60 | Psidium friedrichsthalianum | 6 | 12.5 | 0.58 | 0.12 | 1.45 | 0.47 | 224 | 0.70 | 0.77 |
| 61 | Pterocarpus rohrii | 594 | 30.2 | 0.35 | 0.13 | 1.79 | 6.80 | 240 | 0.75 | 0.33 |
| 62 | Schizolobium parahybum | 47 | 17.8 | 0.43 | 0.14 | 0.68 | 22.99 | 215 | 0.72 | 0.27 |
| 63 | Senna papillosa | 155 | 25.7 | 0.27 | 0.17 | 1.83 | 3.53 | 171 | 0.75 | 0.48 |
| 64 | Sideroxylon nigra | 97 | 11.9 | 0.45 | 0.16 | 1.91 | 1.49 | 265 | 0.75 | 0.78 |
| 65 | Siparuna andina | 101 | 21.1 | 0.25 | 0.19 | 0.94 | 3.44 | 246 | 0.75 | 0.43 |
| 66 | Solanum rudepanum | 213 | 17.0 | 0.26 | 0.20 | 0.75 | 5.05 | 189 | 0.74 | 0.41 |
| 67 | Solanum umbelatum | 93 | 18.4 | 0.27 | 0.22 | 0.67 | 0.90 | 243 | 0.70 | 0.44 |
| 68 | Tabernamontana alba | 65 | 12.6 | 0.25 | 0.25 | 1.23 | 1.70 | 206 | 0.66 | 0.39 |
| 69 | Terminalia amazonia | 22 | 10.0 | 0.45 | 0.23 | 1.89 | 1.03 | 220 | 0.71 | 0.45 |
| 70 | Thevetia ahouai | 81 | 11.6 | 0.18 | 0.50 | 1.01 | 0.57 | 220 | 0.73 | 0.28 |
| 71 | Trema laxiflora | 42 | 15.7 | 0.34 | 0.20 | 0.80 | 1.07 | 165 | 0.68 | 0.42 |
| 72 | Trema micrantha | 55 | 21.3 | 0.29 | 0.18 | 0.80 | 1.54 | 193 | 0.71 | 0.38 |
| 73 | Trichospermum mexicanum | 85 | 16.7 | 0.38 | 0.17 | 1.35 | 2.07 | 206 | 0.67 | 0.25 |
| 74 | Vatairea lundellii | 351 | 19.0 | 0.39 | 0.11 | 2.13 | 11.41 | 235 | 0.70 | 0.63 |
| 75 | Vernonia patens | 30 | 15.7 | 0.33 | 0.17 | 1.06 | 0.74 | 193 | 0.74 | 0.41 |
| 76 | Vismia camparaguey | 339 | 10.9 | 0.39 | 0.21 | 1.55 | 2.60 | 184 | 0.73 | 0.41 |
| 77 | Vochysia guatemalensis | 83 | 10.8 | 0.29 | 0.27 | 1.14 | 1.62 | 194 | 0.71 | 0.36 |
| 78 | Xylopia frutescens | 7 | 15.0 | 0.45 | 0.20 | 1.14 | 0.31 | 259 | 0.62 | 0.51 |
| 79 | Zanthoxylum caribaeum | 580 | 16.4 | 0.36 | 0.20 | 1.51 | 6.13 | 191 | 0.74 | 0.59 |

| | Species | LA | SLA | LDMC | LT | sFtP | PL | $F_0$ | Qy | WD |
|---|---|---|---|---|---|---|---|---|---|---|
| 80 | Zanthoxylum kellermanii | 691 | 10.8 | 0.45 | 0.22 | 1.15 | 9.87 | 160 | 0.71 | 0.41 |
| 81 | Zanthoxylum procerum | 488 | 13.9 | 0.43 | 0.14 | 2.46 | 8.69 | 250 | 0.74 | 0.50 |
| 82 | Zuelania guidonia | 117 | 9.9 | 0.40 | 0.25 | 1.47 | 0.94 | 169 | 0.73 | 0.53 |

**Appendix 2**

*Correlation matrix of the nine functional traits; upper-right half of the table represent the community weighted mean trait values per plot, lower half of the table represent the simple community average trait values per plot (n= 11). Shown are the Pearson correlation coefficient and the significance (2 tailed), \* indicates that a spearman test was used due to the lack of normally distributed data, for LT community weighted mean is transformed with the natural logarithm. Bold results are significant correlations. LA = Leaf Area; SLA = Specific Leaf Area; LDMC = Leaf Dry Matter Content; LT = Leaf Thickness; sFtP = specific Force to Punch; PL = Petiole Length; $F_0$ = instantaneous chlorophyll fluorescence; QY = Quantum Yield; WD = Wood Density*

| | LA | SLA | LDMC | *ln LT | sFtP | PL | $F_0$ | QY | WD |
|---|---|---|---|---|---|---|---|---|---|
| LA | - | .185 | .539 | -.400 | .278 | **.879** | -.201 | -.451 | .432 |
| | | .585 | .087 | .223 | .407 | **.000** | .552 | .164 | .184 |
| SLA | .067 | - | -.099 | -.545 | .169 | .416 | .238 | -.252 | -.371 |
| | .845 | | .772 | .083 | .619 | .204 | .480 | .454 | .261 |
| LDMC | .370 | **-.619** | - | -.464 | .552 | .227 | -.008 | -.475 | **.785** |
| | .263 | **.042** | | .151 | .079 | .502 | .982 | .140 | **.004** |
| LT | -.377 | -.230 | -.424 | - | .036 | -.327 | .273 | .455 | .073 |
| | .253 | .495 | .194 | | .915 | .326 | .417 | .160 | .832 |
| sFtP | **-.823** | -.290 | .000 | .028 | - | .159 | **.706** | -.362 | **.630** |
| | **.002** | .388 | .999 | .934 | | .641 | **.015** | .274 | **.038** |
| PL | **.939** | .298 | .161 | -.329 | **-.870** | - | -.055 | -.448 | .094 |
| | **.000** | .373 | .636 | .323 | **.000** | | .873 | .167 | .784 |
| $F_0$ | **-.926** | -.159 | -.281 | .260 | **.871** | **-.850** | - | -.235 | .121 |
| | **.000** | .641 | .402 | .441 | **.000** | **.001** | | .486 | .724 |
| *QY | -.373 | .164 | -.282 | .191 | .145 | -.236 | .364 | - | -.017 |
| | .259 | .631 | .401 | .574 | .670 | .484 | .272 | | .961 |
| WD | **-.811** | -.439 | .073 | .170 | **.836** | **-.832** | **.911** | -.042 | - |
| | **.002** | .177 | .832 | .617 | **.001** | **.002** | **.000** | .902 | |

## Chapter 3: Functional and species diversity in tropical wet forest succession

**Abstract**

Although functional diversity, 'those components of biodiversity that influence how an ecosystem operates or functions', receives ample theoretical attention, published empirical data are scarce. We analyse functional diversity changes in tropical secondary forest succession in Chiapas, Mexico. Additionally we test the ability to detect differences in functional diversity among sites when only species diversity data are available. Functional diversity is used as the combined effect of all traits measured, but since ecosystem functions are linked to (few) specific traits, we also analyze the functional diversity two individual traits (SLA and WD) that are important for primary production and carbon sequestration. Time after abandonment did not directionally change any of the diversity measures, forest structure proved a better predictor of diversity changes. Non-weighted measures of diversity (based on presence/absence) increased logarithmically with stand basal area whereas indices weighted for species abundances lacked pattern with succession in our secondary forest sites. Functional diversity (non-weighted; all traits) increases linearly with species richness, indicating that functional redundancy may not occur in our secondary forest sites. Abundance weighted functional diversity (all traits), crucial for studying ecosystem processes (mass ratio hypothesis), increases exponentially with species richness.

Our results provide empirical evidence that species richness and species diversity are likely to underestimate the (weighted) functional diversity in a site when a range of traits (9 in this study) is considered. Instead when a single trait is included in the functional diversity measure, species diversity may underestimate (in case of SLA) or overestimate (in case of wood density) the functional diversity. This study substantiates the need for careful consideration of the functional trait(s) to be included in studies targeting the relation between functional diversity and ecosystem processes because the choice of traits will influence results when comparing sites.

Keywords: Functional diversity; Species diversity; Ecosystem functioning; Ecosystem processes; Secondary forests; Chronosequence; Chiapas; Mexico.

**Introduction**

Functional diversity has been defined as the value, range, and distribution of functional traits in a given ecosystem (Lavorel *et al.* 2007; Diaz *et al.* 2007a) or as those components of biodiversity that influence how an ecosystem operates or functions (Tilman 2001). Many researchers recognize that functional diversity is more important to local scale ecosystem functioning than taxonomic diversity (Grime 1998; Tilman 2001; Naeem & Wright 2003; Petchey 2004; Petchey et al. 2004; Hooper *et al.* 2005) and may be the most important biodiversity measure (Diaz & Cabido 2001). Functional diversity may be a better indicator of ecosystem stability and productivity than species diversity is (Tilman 2000; Díaz *et al.* 2007b). Indeed, a higher functional diversity is, for example, related to resistance against invasive species (Dukes 2001; Funk *et al.* 2008), and to increased efficiency in nutrient and light capture (Tilman *et al.* 1997) and thus to increased productivity (Nyström & Folke 2001). Functional diversity may affect short-term ecosystem resource dynamics and long-term ecosystem stability (Díaz & Cabido 2001; Walker *et al.* 1999; Nyström & Folke 2001). Theory

predicts that higher functional diversity increases primary production and nutrient cycling through the effect of resource use complementarity among species in the community (Hooper 1998; Petchey 2003).

Several indices have been proposed for calculating functional diversity (Mason *et al.* 2003; Mouillot *et al.* 2005; Pavoine *et al.* 2005; Petchey & Gaston 2006). In current study we used functional diversity as the range of traits based on presence/absence as well as based on species abundances in the community. Functional diversity indices based on presence/absence of species are comparable to functional richness measures (*e.g. Functional Attribute Diversity* of Walker *et al.* 1999; *Functional Diversity* of Petchey & Gaston 2002). Such indices emphasize the full range of functional strategies present, as dominant and rare species equally contribute to functional diversity. The philosophy behind this approach is that each individual species may strongly influence ecosystem functioning and rare species are thought to be important contributors to long-term resilience (Walker *et al.* 1999; Hooper *et al.* 2005). In contrast, when species abundances are used as weightings, the role of dominant species is emphasized (*e.g. FDvar* of Mason *et al.* 2003; *Functional regularity* of Mouillot *et al.* 2005). Such indices are in line with the biomass-ratio hypothesis (Grime 1998; Diaz & Cabido 2001) stating that the dominant species have a greater effect on ecosystem functioning.

Also the number and type of traits included in diversity indices plays a role. Studying ecosystem functioning, the need to include only the trait(s) relevant for the function of interest is stressed (Diaz *et al.* 2007b), therefore we test the effect of single trait functional diversity and functional diversity based on a range of traits. Single trait functional diversity is calculated based on a leaf trait (specific leaf area) and a stem trait (wood density). These traits are chosen because they are some of the most important and commonly studied traits and are of paramount importance for different ecosystem processes (Lavorel & Garnier 2002). SLA has an effect on the processes primary production, carbon and nutrient cycling and litter decomposition (*e.g.* Lavorel & Garnier 2002; Diaz *et al.* 2004; Poorter *et al.* 2009) whereas wood density is a key trait in predictions on biogeochemical ecosystem processes such as carbon sequestration and cycling (*e.g.* Chave *et al.* 2009).

Although functional diversity receives ample theoretical attention, empirical data on how functional diversity varies across environmental and successional gradients is lacking. The current study applies the concept of functional diversity to secondary tropical forest succession and this is, as far as we know, the first study to do so. Because succession reflects community assembly in action, it provides a good opportunity to test how functional diversity varies from very simple, species poor, early successional communities towards more complex, species rich, late successional communities. Functional diversity is expected to increase during early stages of succession after which it may decrease again, due to a peak in diversity, caused by coexistence of pioneer and shade tolerant species at intermediate time after disturbance (the Intermediate Disturbance Hypothesis - Connell 1978, Bongers *et al.* 2009). As our chronosequence includes only relatively early stages of succession, we expect that functional diversity will increase over time.

Also we explored the relation between functional diversity and species diversity. Discussing the relation between functional diversity and species diversity essentially deals with the extent of trait complementarity among species (or oppositely, the extent of

functional redundancy among species) (Petchey & Gaston 2002) and accordingly with the extent in which species diversity can be used as a proxy for functional diversity. Functional diversity is expected to be positively related to species diversity; when one species is added to the ecosystem, it is likely that this species will not only contribute to species diversity but also to functional diversity meaning that the set of functional traits encompassed by this species was not present before. However it has been suggested that species diversity is limited by the regional species pool whereas functional diversity is limited by the local availability of niches (Schmid *et al.* 2002) indicating that both may be non-related. In case of a linear relationship, species richness is a good proxy for functional diversity, which makes detailed and time consuming measurements of functional traits unnecessary. However this linear relation is not commonly found in nature (Díaz & Cabido 2001) and rather partial complementarity (logarithmic) or non-complementarity (no relation) could be found (Schmid *et al.* 2002). A logarithmic relationship between species diversity and functional diversity indicates that species added have functional strategies already represented by other species; functional redundancy. When functional diversity is unrelated to species diversity the role individual species with extreme trait values may be underlying irrespective of the diversity of species (Petchey & Gaston 2002) and, in this case, species diversity is not a good surrogate for functional diversity to reflect on ecosystem functioning.

The distribution of trait complementarity is influenced by the 'effective dimensionality of trait space' where a higher dimensionality allows all species to be functionally equally similar (or dissimilar). A higher dimensionality results in a linear relationship between functional and species diversity where the addition of traits of any species increases functional diversity in the same way as adding any other species to the community. The effective dimensionality of trait space is in turn independently affected by the number of traits included in the matrix where an increasing number of traits increases the effective dimensionality of trait space, and by including species abundances (weighting species differently) which will reduce the effective dimensionality of trait space because it essentially deals with only dominant species (Petchey & Gaston 2002).

We analyze functional and species diversity in a chronosequence (3,5 to 22,5 years of fallow age) based on several functional traits relevant for primary productivity, decomposition, consumption by herbivores, heat exchange and biomass accumulation.

This study evaluates (1) how functional and species diversity changes along a gradient of secondary succession in wet tropical forest in southern Mexico, and (2) to what level species diversity reflects functional diversity. Functional diversity is calculated for all nine traits combined, as well as for SLA and wood density separately. We hypothesize that species and functional diversity increase during early secondary succession, and that functional diversity is positively related to species diversity. Functional diversity for all traits combined may show a different pattern with increasing species diversity than the functional diversity of separate traits because of the effect of statistical averaging.


## Methods
*Research site and species selection*
Research was carried out within the Lacandon region in southeast Mexico (16°01'N, 90°55'W). The climate is warm-humid with an average annual temperature of 25-27°C and an annual rainfall of 3.000 mm, with less than 100 mm per month falling from February

through April (Ibarra-Manríquez & Martínez-Ramos 2002; Breugel *et al.* 2006). The study sites are located around the village of Loma Bonita, an area characterized by small hills and valleys with sandy or limestone soils of low pH (<5.5). Currently 65% of the land area around Loma Bonita is used for agricultural practices (mostly livestock pasture), 14% is mature forest and 21% consist of secondary forests of different ages (Zermeño-Hernández 2008).

The successional chronosequence consists of 11 secondary forest plots (10 x 50 m) with fallow ages between 3.5 and 22.5 years. Plots were established on abandoned corn fields, the main form of agriculture, and plots were in use within one year before abandonment. In each plot all trees with a diameter at breast height (DBH) > 1cm were identified and their diameter was measured.

For each plot those species were selected that make up at least 80% of the importance value (calculated by the relative abundance and the relative density, further explained below) of that plot, as it is suggested that those species determine most of the key ecosystem processes (Garnier *et al*. 2004; Pakeman & Quested 2007). On average 32 species per site were selected, ranging from 9 to 47 species, being 82 species in total. Both dominant and rare species have been selected with an average importance value of 0.036 (3.6%) ranging from 0.0007 (0.07%) to 0.63 (63%).

*Functional traits*

Nine functionally important traits were studied, important for the carbon-, water- and nutrient-balance of plants (Table 1). Leaf traits were measured on 2 sun-lit leaves per individual and 10 replicate individuals per species, except for specific force to punch where only 5 individuals per species were measured. Wood traits are also determined for five individuals per species. Standardized measurement protocols have been followed as suggested by Cornelissen *et al.* (2003).

*Leaf Area* ($cm^2$). Leaf area was calculated by photographing the fresh area excluding the petiole on a light box after which the area was calculated with pixel counting software ImageJ (Rasband 2009).

*Specific Leaf Area* ($m^2\,kg^{-1}$). SLA was calculated as the fresh leaf area divided by the oven dried mass (dried for 48 h at 70°C), excluding the petiole. In the case of composite leaves the smallest photosynthetic unit was taken.

*Leaf Dry Matter Content* ($g\,g^{-1}$). After collection the leaves were placed in water to rehydrate for at least 5 hours after which the fresh weight was determined. Oven-dry weight was divided by the fresh weight to determine the leaf dry matter content.

*Leaf thickness* (mm). Leaf thickness was determined with a digital caliper in the middle of the leaf avoiding the main and secondary nerves.

*Specific Force to Punch* ($MN\,m^{-2}$). The laminas of fresh leaves were punched with a penetrometer built with a flat-end nail attached to the inner part of a syringe and a waterbasin on top. Water was added to the basin until the leaf ruptured. The total weight added was converted into Mega Newton and divided over the breaking surface of the hole (circumference of the nail x thickness of the leaf). This alternative punching method is comparable to cutting and tearing tests and is a good indicator of leaf mechanical resistance (Onoda *et al. submitted*).

*Petiole length* (cm). Determined with a digital caliper or a tape-measure.

*Instantaneous Chlorophyll Fluorescence ($F_0$).* Leaves were dark-adapted for 1 hour after which the fluorescence was measured with a fluorpen (Photon Systems Instruments, FP100) on exposure to light, 455 nm, at a saturating intensity of 3000uE.

*Quantum Yield  ($F_v / F_m$).* The quantum yield is the ratio between the variable fluorescence and maximum fluorescence on exposure to saturating light (455 nm, intensity of 3000uE) after dark adaptation (1 hour). It reflects the proportion of light used in photochemistry or emitted as heat. Measurements were taken with a fluorpen (Photon Systems Instruments, FP100).

*Wood Density* (g cm$^{-3}$). With an increment borer (12" mm Suunto, Finland) a core of wood was extracted up to at least the heart of the tree (firmly attached bark or phloem tissue was considered part of the stem). For some species the stems did not reach sufficient size for this method and for these a slice of the stem was extracted. The wood cores were divided in three parts (across the pith, heartwood and sapwood) and the wood density was measured for these parts separately by dividing the dry weight over the fresh volume. The fresh volume was determined with the water replacement method. Wood densities of these three parts were averaged, to obtain the species specific wood density. This measurement was taken for 32 of the 82 species studied, data on wood density for remaining species were taken from comparable studies in Mexican wet forests in the same location around Chajul (Chiapas), Las Margaritas (Puebla) and Los Tuxlas (Veracruz) (Martínez-Ramos & Bongers *unpublished results*).

*Table 1. The nine traits used to calculate the functional diversity for each of the sites; trait abbreviations, a short description of their functional role, their effect on ecosystem processes and the references used.*

| Functional trait | Response to abiotic conditions | Effect on ecosystem processes | References |
|---|---|---|---|
| Leaf area; LA (cm$^2$) | Light intercepting area, dry matter production, respiration, leaf cooling, gas exchange | Evapotranspiration, net primary productivity | Bazzaz & Picket 1980 Popma *et al.* 1992 |
| Specific Leaf Area; SLA  (m$^2$ Kg$^{-1}$) | Light capture economics, net assimilation rate, relative growth rate, leaf life span, photosynthetic capacity | Net primary productivity, relative productivity rate, consumption by herbivores, decomposition and net mineralisation | Reich *et al.* 1997 Cornelissen *et al.* 1999 Poorter & Garnier 1999 Sterck *et al.* 2006 Poorter & Bongers 2006 Poorter *et al.* 2009 |
| Leaf Dry Matter Content; LDMC (g g$^{-1}$) | Leaf tissue density, level of assimilative compounds and transfer conductance to $CO_2$, construction costs and nutrient retention, against | Decomposition, consumption by herbivores, | Poorter & Garnier 1999 Niinemets 2001 Garnier *et al.* 2004 |

| | mechanical and herbivore damage, tolerance against water limitations | | |
|---|---|---|---|
| Leaf Thickness; LT (mm) | Construction costs, leaf life span, photosynthetic rates per unit leaf area, against mechanical and herbivore damage, gas exchange and leaf cooling | Consumption by herbivores, net primary productivity | Reich *et al.* 1991 Popma *et al.* 1992 Niinemets 2001 Díaz *et al.* 2004 Onoda *et al.* submitted |
| Specific Force to Punch (MN m$^{-2}$) | Leaf strength, survival of herbivory and physical damage, persistence, leaf lifespan | Net primary productivity, relative productivity rate, consumption by herbivores, decomposition and net mineralisation | Cornelissen *et al.* 1999 Onoda *et al.* submitted Díaz *et al.* 2007b Poorter *et al.* 2009 Kitajima & Poorter 2010 |
| Petiole length; PL (cm) | Light capture efficiency | Evapotranspiration, net primary productivity | Black 1960 Takenaka 1994 |
| Instantaneous chlorophyll fluorescence; $F_0$ (dimensionless) | Ability to dissipate heat, resistance against overheating, respiration, photosynthetic performance | Heat exchange, net primary productivity | Maxwell & Johnson 2000 Baker & Rosenqvist 2004 |
| Quantum Yield; QY (dimensionless) | Effectiveness of photosystem II, $CO_2$ assimilation, stress tolerance, photosynthetic performance, leaf nutrient levels | Heat exchange, net primary productivity, consumption by herbivores | Maxwell & Johnson 2000 Parkhill *et al.* 2001 Baker & Rosenqvist 2004 Dos Santos *et al.* 2006 |
| Wood Density; WD (g cm$^{-3}$) | Construction costs, growth rate, stem vulnerability, mortality rate | Accumulation or standing biomass | Augspurger & Kelly 1984 van Gelder *et al.* 2006 Poorter *et al.* 2008 Chave *et al.* 2009 |

*Calculating species diversity and functional diversity*
Species diversity and functional diversity indices were calculated with and without weighting for species abundance (Table 2). The importance value is used to reflect abundance of

species, calculated as: $(BA_i/BA_{total} + D_i/D_{total})/ 2$, where $BA_i$ is the basal area of the $i^{th}$ species and, $D_i$ is the density of the $i^{th}$ species and total reflects the total of individuals in the plot. Weighting with this combined measure of density and basal area was chosen because only basal area would bias towards individuals that have the potential to grow a large basal area. We expect that ecosystem processes may be influenced by species with large numbers of individuals, just as by individuals with large biomass.

To test whether species diversity served as a proxy for functional diversity both weighted and non-weighted measures for species and functional diversity were used. Species richness is used as an unweighted species diversity measure, the simple accumulated number of species that are sampled in the sites accounting for at least 80% of the importance value of the forest plot (DBH > 1 cm). A commonly used weighted species diversity measure is Shannon Weaver diversity, this includes the relative importance of species in the formula (Shannon & Weaver 1949).

For functional diversity similarly two types of measures are analyzed, first based on the range of functional profiles present in the sites ('functional richness') and secondly the range weighted for species' relative abundances, hence drawn towards dominant species. Functional diversity was calculated using the fDiversity software program based on Euclidean distance and average linkage (Casanoves *et al.* 2008). We used the measure 'multiplicative extended functional diversity' (Pla *pers. comm.*) based on a dissimilarity matrix of trait-values for all species in the study (Petchey & Gaston 2006). The matrix is loaded with the species' relative abundances and subsequently the multiplicative extended weighted functional diversity is computed as the total branch length of the functional dendrogram of the community multiplied by the squared species number. For functional diversity based on presence/absence of species all abundances in the input file are set at 1.

Functional diversity was calculated for all 9 traits together and also for two key individual traits separately: specific leaf area and wood density.

*Table 2: Overview of the diversity indices used to describe the secondary forest communities, their method of calculation and the references used.*

| Diversity index; abbreviation | Method of calculation | References |
|---|---|---|
| Species number; SD | Total number of species sampled in the site | |
| Shannon Weaver diversity, weighted for species abundances; wSD | $wSD = - \sum_{i=1}^{s} p_i \ln p_i$<br><br>$p_i$ = importance value of species i<br>S= total number of species<br>N= total importance value captured with species sampled<br>Calculated in fDiversity | Shannon & Weaver 1949<br>Casanoves *et al.* 2008 |
| Functional diversity based on presence/ absence; FD | Distance matrix based on species-trait matrix. FD= total branch length of the functional dendrogram * species number$^2$<br>Calculated in fDiversity | Petchey & Gaston 2006<br>Pla *et al.* 2008<br>Pla *pers. comm.*<br>Casanoves *et al.* 2008 |
| Functional diversity | Distance matrix based on species-trait | Petchey & Gaston 2006 |

| weighted for species abundances; wFD | matrix, matrix loaded by abundances. wFD= total branch length of the functional dendrogram * species number$^2$ Calculated in fDiversity | Pla *et al.* 2008 Pla *pers. comm.* Casanoves *et al.* 2008 |
|---|---|---|

*Statistical analysis*

All statistical analysis were carried out using SPSS 17.0.

Regression analysis and Pearson correlations were carried out to test species and functional diversity change in time since abandonment and stand basal area. Also for relating functional diversity with species diversity regression analysis was performed. The best model fit is tested with single regression curve estimation in SPSS. For a better description of the trends, the bivariate relationships were additionally analyzed using simple (two-parameter) linear, logarithmic and exponential models.

**Results**

*1. Species and functional diversity during secondary succession*

None of the diversity measures showed a significant relationship with age of the secondary forest (Table 3). When basal area was used instead as a reflection of structural community development during succession, logarithmic increases were found for species richness ($R^2$ = .55 p= .009), (non-weighted) functional diversity of all traits combined ($R^2$ = .53 p=.011) and (non-weighted) functional diversity of wood density values ($R^2$ = .65 p=.003) (Figure 1). SLA did not show a significant response. In contrast, diversity measures that included weighting for species abundances (Shannon Weaver, weighted functional diversity of all traits together, weighted functional diversity of SLA and of wood density) did not increase with increasing stand basal area (Table 3).

*Table 3. Pearson correlations of diversity changes along a successional gradient. Non-weighted species diversity (SD/ species richness), species diversity weighted for species relative abundances (wSD), non-weighted functional diversity (FD), and functional diversity weighted for species abundances (wFD). Functional diversity is based on all nine traits (FD (all)) or based on a single trait (FD (SLA) and FD (WD)). Given are the Pearson correlation coefficient and the significance levels. Bold results are significant correlations, and have also been explored with curve fitting analysis (Figure 1).*

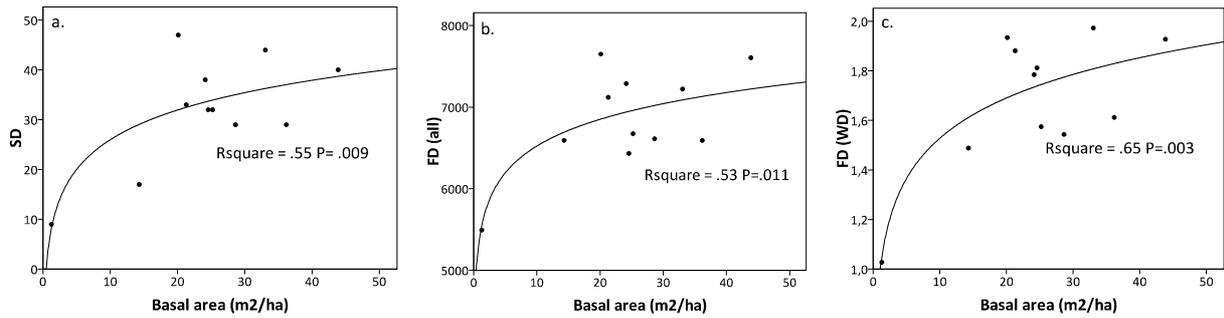| Diversity | Time | Basal area |
|---|---|---|
| SD | R= .29 p= .386 | **R= .66 p= .028** |
| wSD | R= -.09 p= ,786 | R= .19 p= .570 |
| FD (all) | R= .11 p= .756 | **R= .62 p= .043** |
| FD (SLA) | R= -.07 p= .837 | R= -.24 p= .473 |
| FD (WD) | R= .30 p= .369 | **R= .67 p= .023** |
| wFD (all) | R= -.27 p= .416 | R= .46 p= .151 |
| wFD (SLA) | R= .38 p= .244 | R= .39 p= .238 |
| wFD (WD) | R= -.04 p= .913 | R= .49 p= .130 |

*Figure 1: Species and functional diversity changes during secondary succession where stand basal area is used to reflect vegetation changes along a successional gradient. Of the three regressions used (linear, logarithmic and exponential), the best fitting model is shown. a. species richness (SD) as a logarithmic function of stand basal area; b. Functional richness (non-weighted functional diversity; FD) based on all traits as a logarithmic function of stand basal area; c. Functional richness based on wood density (FD (WD)) as a logarithmic function of stand basal area.*

*2. Functional diversity related to species diversity*

We examined the ability to use species diversity as a proxy for functional diversity by analyzing the shape of the relation of functional diversity plotted against species diversity. Non-weighted functional diversity ('functional richness') is linearly related to species richness (Figure 2a: $R^2$ = .80 p= .000). We describe this relation as linear because it is the simplest model, *although the exponential model had the same fit*. Single trait-based functional richness showed no significant trend against species richness for SLA (Figure 2b), but showed a logarithmic relation for wood density (Figure 2c: $R^2$ = .88 p= .000). Functional diversity weighted for species abundances is exponentially related to species richness (Figure 2d: $R^2$ = .44 p= .027), which is also the case when functional diversity is analyzed for the single traits SLA (Figure 2e: $R^2$ = .72 p= .001) and wood density (Figure 2f: $R^2$ = .72 p= .001).

When weighted functional diversity is plotted against weighted species diversity (Shannon index), an exponential relationship is found for all trait combined (Figure 2g: $R^2$ = .40 p= .036) and for SLA (Figure 2h: $R^2$ = .52 p= .013) while a logarithmic relationship is found for wood density (Figure 2i: $R^2$ = .50 p= .015).
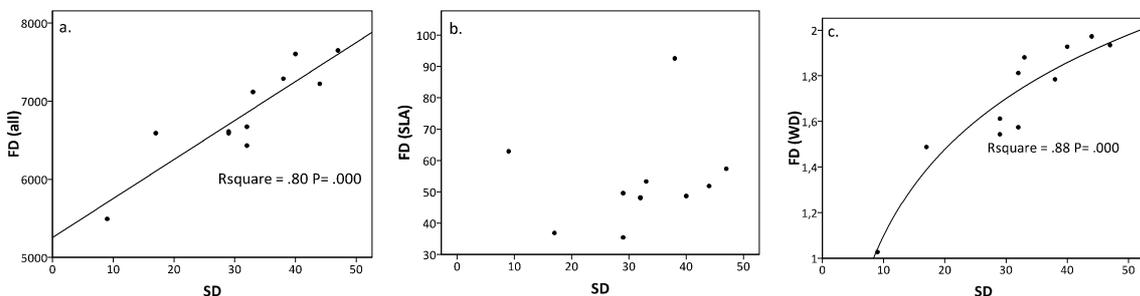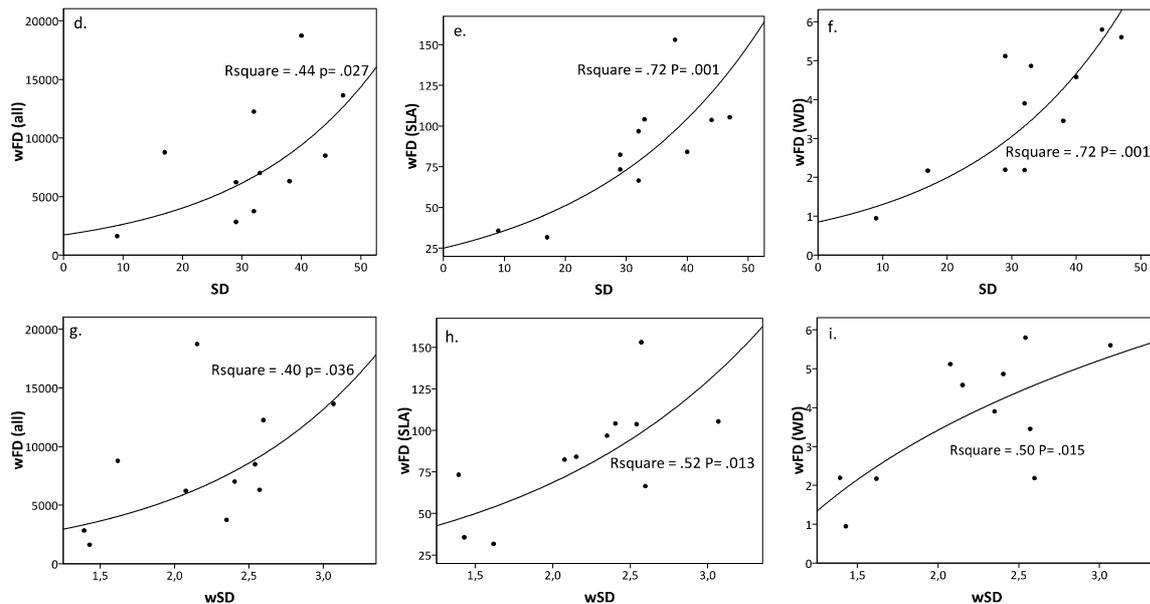
*Figure 2: Functional diversity as a function of species diversity indices. Left, middle and right column illustrate functional diversity measures based on all traits, SLA and wood density respectively. Upper row illustrates the relations between non-weighted species diversity (SD) and non-weighted functional diversity (FD), middle row shows the relations between non-weighted species diversity (SD) and weighted functional diversity (wFD) and the lower row of graphs illustrate weighted functional diversity (wFD) as a function of weighted species diversity (wSD). The best fitting curves (tested for linear, logarithmic and exponential) are depicted in the figures with the $R^2$ and significance levels.*

## Discussion

*1. Do species and functional diversity increase in a predictable way during secondary succession?*

We predicted that functional and species diversity both increase during secondary succession. We found that only non-weighted species and functional diversity increase significantly and that these relate to stand basal area rather than with time after abandonment.

Lack of diversity patterns with time since abandonment of the forest sites in our chronosequence may be explained by the variation in diversity trajectories across the sites, where the assumption that all sites follow the same successional trajectory is not always justified (Guariguata & Ostertag 2001; Chazdon *et al*. 2007). Basal area proved a better predictor of diversity changes in our sites than fallow age which is more a compound variable reflecting stand structure, history of land use and within plot environmental heterogeneity at a certain point during succession (Breugel *et al.* 2006). Stand basal area is a proxy for community structure which is seen as an important driver of succession because structure shapes the local environment which filters for traits successfully adapted to local conditions (Lebrija-Trejos *et al.* 2010).

Only the diversity indices that do not include for species abundances significantly increase with basal area. The curves are best described by a logarithmic model meaning that during early structural development new species are recruited that are not yet represented in the site, and that have functional strategies that are complementary to the ones already

present. At later stages of structural development the increases in species richness and functional richness slows so increases in basal area are now mainly driven by either addition of individuals that are already represented in the site (functionally and from a species perspective) and/or by existing individuals increasing in size. It is likely that the saturating relationship can be attributed to both processes. The first mechanism can be understood by the chance that a 'new' species or functional type dispersing into the site decreases as the number of species is higher (Schmid *et al.* 2002). The second mechanism is supported by the notion that during secondary succession biomass shows a saturating increase due to biomass being accumulated in fewer but bigger individuals (Brown & Lugo 1990).

No increases take place in any of the weighted diversity variables. This can be explained by the fact that some stands are dominated by few species that show highly erratic abundance patterns over time. For example Breugel *et al.* (2007) in the same area show mass mortality of the pioneer *Ochroma pyramidale* in some sites. Also the oldest plot with an age of 22.5 years after abandonment is strongly dominated by *Vochysia guatemalensis*, making up for 63% of the site's importance value. Such site-specific observations of demographic rates may explain the loss of signal when weighted diversity is analyzed over a chronosequence.

*2. Is species diversity a good predictor of functional diversity?*

As expected functional diversity generally increases with species diversity, and we also found that the shape of this relationship depends on the functional diversity index used (non-weighted or weighted), and on whether all traits or single trait values are used.

When non-weighted functional diversity is plotted against non-weighted species diversity we find a strong linear relation (Figure 2a). Díaz and Cabido (2001) state that a linear relationship between functional richness and species richness can theoretically only occur in two scenarios characterized by a high intraspecific homogeneity of niche space. Firstly, when there is a random occupation of niche space and where species have limited functional overlap. Secondly, when there is a uniform occupation of niche space and where no functional overlap of species occurs. Although they argue that neither of these cases is likely to occur in nature, where rather some 'lumpiness' of species in functional trait space is found, our linear relationship suggests that all species are equally functionally complementary. This means that each species added to the system also represents a functional profile that was not present before, *i.e.* the addition of any species to the community causes a similar increase in FD (all) to that caused by any other species and replacing any species with another one does not change the relationship found. Putting this in terms of Petchey and Gaston (2002), the linear relationship is an effect of high effective dimensionality of trait space allowing species to differ similarly. From this linear relation we can also conclude that in our wet tropical secondary forests functional redundancy was not identified. Functional redundancy is implied when increased species diversity causes little change in functional diversity, and means that new species overlap in functional traits with resident species. Although functional redundancy may be criticized as being incompatible with coexistence (Loreau 2004), others emphasize its importance: functional redundancy rather than functional diversity is what maintains or restores ecosystem functioning under changing environmental conditions (resilience) or disturbance events (Walker *et al.* 1999; Díaz & Cabido 2001).

Analyzing the functional diversity of a single trait reduces the effective dimensionality of the functional diversity so that the identity of species is emphasized and an increase in functional diversity depends much on the trait value of the particular species added (Petchey

& Gaston 2002). We found that the (non-weighted) functional diversity of SLA was not related to species richness (Figure 2b) whereas wood density showed a logarithmic relation (Figure 2c). The lack of pattern in the case of SLA indicates that coexisting species differ in their extent of functional complementarity of SLA values, regardless of the number of species actually present. Functional diversity of wood density increases logarithmically with species richness indicating that the relative similarity in wood density increases with higher species richness. The saturating increase of single-trait functional diversity when plotted against the number of species is typical when few traits separate species. It signifies that some pairs of species complement each other in wood density values whereas others do not (partial complementarity) and with increasing species number the chances increase that the wood density value added is already present in the community. This influence of compositional difference between sites is caused by the reduction in effective dimensionality of trait space by reducing the number of traits to a single-trait diversity (Petchey & Gaston 2002). The saturating increase indicates that there is redundancy in species' contribution to the functional diversity based on wood density (FD (WD)). This does not unambiguously allow us to conclude that functional redundancy occurs in the secondary forest sites studied because the functional analogues based on wood density may still encompass a large variety of other traits ensuring long-term stability of community structure. This was also argued to explain the similar growth forms in South African fynbos where within the functional type of growth form still large variation in regeneration traits existed (Cowling *et al.* 1994).

According to the biomass ratio hypothesis (Grime 1998) the functional characteristics of the abundant species largely determine the effect on ecosystem processes, stressing the need to use a weighted measure of functional diversity in case ecosystem processes are the main interest of study. Plotting species richness against weighted functional diversity is relevant to assess whether the easily obtainable measure of species number could serve as a proxy for such weighted measure of functional diversity. However we found that weighted functional diversity (for all traits) is exponentially related to species richness (non-weighted), indicating that species richness is likely to underestimate functional diversity. This is the case for weighted functional diversity based on all traits, as well as for weighted functional diversity based on SLA and wood density separate. Because species abundances are incorporated the effective dimensionality of trait space is reduced, and even further when SLA and WD are analyzed separately because of the reduction in number of traits included. This reduction of trait dimensionality brings community composition to the foreground, and highlights the functional identity of the dominant species (like strong functional types), allowing functional diversity to further differentiate between communities when species richness no longer differentiates. The exponential increase in functional diversity with increasing species richness illustrates that functional diversity continues to increase while species number stagnates. Because the functional diversity is weighted for species abundances this can mean two things: either the species abundances in the site are becoming more evenly distributed, or the co-occurring species become more functionally distant from each other due to increased niche differentiation.

When weighted functional diversity is plotted against weighted species diversity both axes have a reduced effective dimensionality caused by the unequal weighting of species. The exponential relation for functional diversity based on all traits indicates that species diversity, like species richness, will probably underestimate functional diversity and ecosystem functioning. Higher effective dimensionality of functional diversity compared to

species diversity is caused by the maximum of 9 ways in which functional diversity can differentiate in community structure (the number of traits when all traits are independent), instead of just one, species abundances, regardless of their functional characteristics. Interestingly, when the effective dimensionality of functional diversity is further reduced to one single trait, we find that SLA and wood density respond differently. For diversity in SLA values the effect of species composition and species identity is high (exponential curve) so that species diversity is likely to underestimate the functional diversity of SLA values. Therefore, when species diversity is used to conclude on the diversity of litter decomposition rates, for studies on forest soil fertility for example, species richness is likely to underestimate forest functioning. On the contrary, wood density shows a logarithmic curve with species diversity. This suggests that species differences in wood density are so limited (partial complementarity) that species diversity is likely to overestimate the functional diversity of wood density. So for example studies interested in carbon cycling will not be served with species diversity as an indicator of forest functioning in the carbon cycle. The exponential curve for weighted functional diversity of SLA and the logarithmic curve of wood density against species diversity indicates that functional complementarity for SLA is much higher than for wood density in the secondary forests studied. Therefore the choice of traits may significantly influence the outcome when comparing functional diversity among sites.


**Conclusions**
Species and functional richness increase logarithmically with structural development of succession. Results indicate that richness already starts leveling off at early stages of succession (under 24 years of abandonment).
Species richness is a good predictor of functional richness (non-weighted) in our secondary forest sites. However, as stressed by the biomass ratio hypothesis (Grime 1998) functional diversity needs to be weighted by species abundances when ecosystem processes is of interest since this is mainly determined by the functional characteristics of the dominant species.
Our results provide empirical evidence that species richness and species diversity are likely to underestimate the weighted functional diversity in a site when a large range of traits is considered. Instead when a single trait is included in the functional diversity measure, species diversity may underestimate (in case of SLA) or overestimate (in case of wood density) the weighted functional diversity. Therefore careful consideration of the traits required to capture the ecosystem process of interest is essential (Díaz & Cabido 2001; Petchey & Gaston 2002) because the number and nature of traits significantly determine the outcome of studies that compare functional diversity across sites.

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# Chapter 4: Functional diversity as a tool in predicting community assembly processes

## Abstract

Deterministic theories on community assembly predict that species presence is largely determined by niche differences, thus by plant functional adaptations. This implies that habitat filtering as an assembly force selects for species in a community to be functionally similar whereas competition favours co-occurring species to be functionally dissimilar to allow them to coexist on the same resource. We predicted that along a gradient of secondary succession, there is a shift in assembly processes where in early stages successful species are characterized by functional similarity (through habitat filtering) and later stages successful species are instead functional dissimilar (through competitive exclusion). We used functional diversity based on presence/absence for the overall community to compare to functional diversity weighted for abundances reflecting the diversity in functional traits of dominant or successful species. Using functional diversity for implying assembly processes has not been done before and our results indicate that the hypothesized shift does indeed occur in secondary forests in the study region. When we analyze all traits combined the dominant species become relatively more functionally dissimilar compared to all species present as the number of species in the plot increases. When the traits are analyzed separately we find that most traits (6 out of 9) show strong significant increases in functional dissimilarity of dominant species indicating that during succession these traits are central to adaptation strategies in an environment characterized by increasing competitive pressure on resources during succession. Our findings show that as more species enter the forests, there is a shift in assembly force from habitat filtering to competitive exclusion determining which species become abundant.

Keywords: Functional diversity; Secondary forests, Chronosequence, Community assembly processes; Habitat filtering; Competition; Tropical forests, Chiapas, Mexico

## Introduction

Community assembly processes are at the heart of community ecology. The debate on how communities are assembled is controversial, however (*e.g.* Hubbel 2001; Chase 2003). Likewise, there is much controversy on species coexistence and maintenance of biodiversity (*e.g.* Wright 2002). Deterministic theories predict that species niche differences create differential responses to environmental conditions and interspecific interactions. Part of this is the environmental filtering hypothesis (Weiher & Keddy 1995) predicting that species are functionally adapted to grow and survive under specific environments and as a result of this species with similar niche requirements are found in similar habitats. The competitive exclusion hypothesis is another example, with it's roots in already in early work on the evolution theory. When resource are limited, competition shapes coexistence via the process of niche differentiation or competitive exclusion and this would lead to co-occurring species adapted to differing niche requirements (*e.g.* Hardin 1960; Grime 1973). Hubbell (2001) instead criticizes the concept of niches and suggests that biodiversity is controlled by neutral drift of species abundances where all species have equal chances of becoming abundant, despite being functionally non equivalent.

This study analyzes community assembly processes in wet tropical forests along a gradient of secondary succession. Secondary succession is ongoing community assembly and therefore provides a perfect setting to test species coexistence from a functional perspective. If indeed species successfulness is shaped by their functional characteristics (as predicted by deterministic theories) functional information on plant species enhances understanding on community assembly. We compared the relative contribution of dominant species (functional diversity weighted for abundances) to the overall functional diversity of a site (functional diversity where all species are equally weighted) to get an idea of what ecological drivers have shaped the community observed. Comparing functional diversity with and without weighting for abundances, gives insights into the functional similarity or dissimilarity of dominant species. This method allows answering the question: 'what ecological force (competitive exclusion or habitat filtering) has selected some of the species present to become dominant?' by addressing whether dominant species are more similar or more dissimilar compared to all species present. This is different from studies where the species in the community are compared to the species pool, testing assembly processes that select species from the pool to be present or not (Figure 1) (*e.g.* Cornwell *et al.* 2006).

When weighted functional diversity (wFD; weighted for species abundances) is lower than non-weighted functional diversity (FD; based on species presence/absence), it indicates that dominant species are functionally similar compared to the overall diversity present. This is a sign that habitat filtering may be the most important driver of community assembly resulting in the selection of specific plant traits that are well adapted to the environment, also referred to as functional (or phenotypic) attraction (Webb *et al.* 2002; Kraft *et al.* 2008)(Figure 2a). Instead, when wFD (weighted for species abundances) is higher than FD (based on presence/absence), it indicates that there is selection for dominant species to be less functionally similar. This can be explained by competition (niche differentiation) as a driver of community assembly, resulting in functional repulsion to allow coexistence of species in a competitive environment (Webb *et al.* 2002; Kraft *et al.* 2008) (Figure 2b). The wFD/FD ratio, as a measure of functional dissimilarity of dominant species, is tested against different gradients that are assumed to reflect different aspects of succession; age, stand basal area and species number.

In time since abandonment the relative importance of competition is expected to increase, in line with the colonization-competition hypothesis (or spatial competition hypothesis). After disturbance, the chance strong competitors have colonized the sites increases in time because of the trade-off between either being a good colonizer or being a good competitor (Levins & Culver 1971; Tilman 1994; Yu & Wilson 2001; Clark *et al.* 2004). The shift in assembly processes predicted reflects an underlying hypothesis on resource limitation; where in early succession a certain resource is abundant, species successfulness will depend on how successfully it can use the resource for growth and reproduction. As more species enter the system that particular resource may become limited reflecting the need for differentiated strategies to obtain this resource, indicated by functional repulsion (dissimilarity) of co-occurring species through competition. By testing the trend in the ratio between weighted and unweighted functional diversity against succession, we may find that some traits clearly reflect increasing competition for resources whereas other do not. This could enhance our understanding on what traits play an important role in adaptation to changing conditions during secondary succession.

The objective of this study is to evaluate how functional diversity can help in analyzing drivers of community assembly. For this we studied the functional characteristics of plant communities along a chronosequence in wet tropical forest in southern Mexico. We specifically address the questions:

How does the ratio ln(wFD/FD) (the relative dissimilarity of dominant species) change with succession, (where the variables used for succession are time since abandonment, stand basal area and species richness) and how does this reflect changing community assembly processes along a successional gradient? We hypothesized that the ratio ln(wFD/FD), will increase in time since abandonment, indicating an increased role of competition as assembly force and a decreased role of habitat filtering.
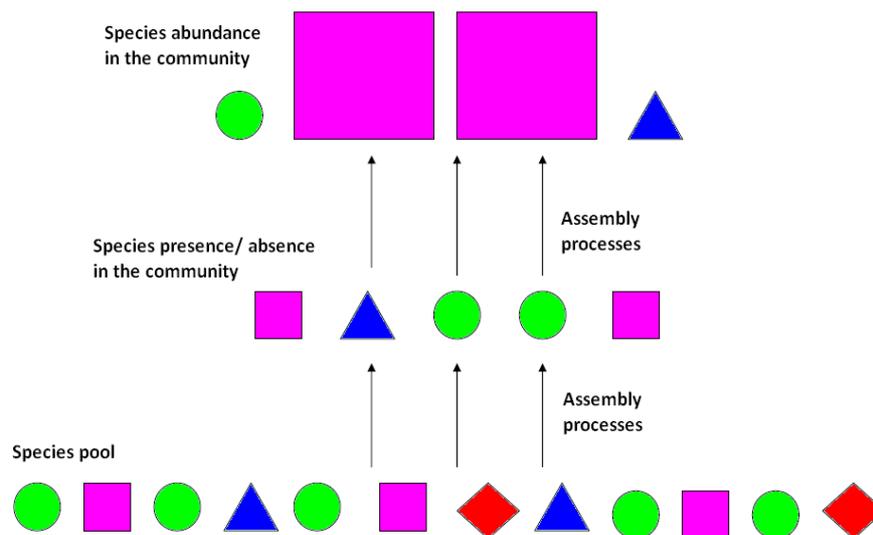


*Figure 1: Illustration of the different steps of assembly processes that take place shaping a community. This study focuses at the assembly processes that shape the species present to become abundant. Another way of analyzing assembly processes is by comparing the species present in the stand to the species pool in the region, this is not taken into consideration in our study. Large objects are individuals with large abundances, different shapes/colors indicate different functional strategies.*



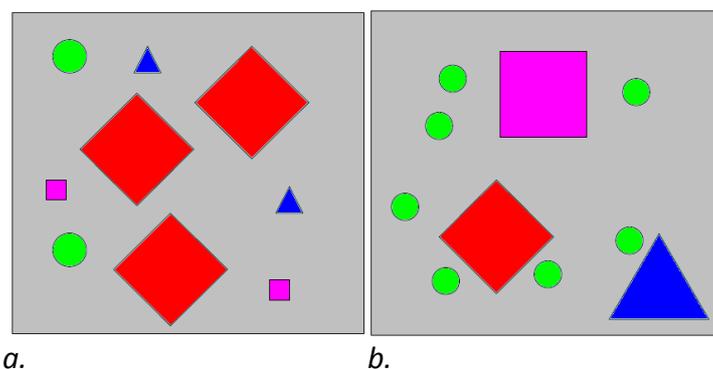a.                                    b.

*Figure 2: a. FD including abundances is lower than FD based on presence/absence, meaning that dominant species are functionally similar. This functional attraction of dominants is an indicator of habitat filtering as an important driver of community assembly. b. FD including abundances is higher than FD based on presence absence. The functional repulsion of dominants is an indicator of competitive exclusion as a major driver of community assembly.*

*Large objects are individuals with large abundances, different shapes/colors indicate different functional strategies.*


**Methods**
*Research site and species selection*
Research was carried out within the Lacandon region in southeast Mexico (16°01'N, 90°55'W). The climate is warm-humid with an average annual temperature of 25-27°C and an annual rainfall of 3.000 mm, with less than 100 mm per month falling from February through April (Ibarra-Manríquez & Martínez-Ramos 2002; Breugel *et al.* 2006). The study sites are located around the village of Loma Bonita, an area characterized by small hills and valleys with sandy or limestone soils of low pH (<5.5). Currently 65% of the land area around Loma Bonita is used for agricultural practices (mostly livestock pasture), 14% is mature forest and 21% consist of secondary forests of different ages (Zermeño-Hernández 2008).
The successional chronosequence consists of 11 secondary forest plots (10 x 50 m) with fallow ages between 3.5 and 22.5 years. Plots were established on abandoned corn fields, the main form of agriculture, and plots were in use within one year before abandonment. In each plot all trees with a diameter at breast height (DBH) > 1cm were identified and their diameter was measured.
For each plot those species were selected that make up at least 80% of the importance value (calculated by the relative abundance and the relative density, further explained below) of that plot, as it is suggested that those species determine most of the key ecosystem processes (Garnier *et al*. 2004; Pakeman & Quested 2007). On average 32 species per site were selected, ranging from 9 to 47 species, being 82 species in total. Both dominant and rare species have been selected with an average importance value of 0.036 (3.6%) ranging from 0.0007 (0.07%) to 0.63 (63%).


*Functional traits*
Nine functionally important traits were studied. Leaf traits have been measured on 2 (sun-lit) leaves per individual and 10 replicate individuals per species, except for specific force to punch where only 5 individuals per species were assessed. Wood traits are also based on five individuals per species. Standardized measurement protocols have been carried out as suggested by Cornelissen *et al.* (2003).
*Leaf Area* (cm$^2$). Leaf area was calculated by photographing the fresh area excluding the petiole on a lightbox after which the area was calculated with pixel counting software ImageJ (Rasband 2009).
*Specific Leaf Area* (m$^2$ Kg$^{-1}$). SLA was calculated as the fresh leaf area divided over the oven dried mass (dried for 48 h at 70°C), excluding the petiole. In the case of composite leaves the smallest photosynthetic unit was taken.
*Leaf Dry Matter Content* (g g$^{-1}$). After collection the leaves were placed in water to rehydrate for at least 5 hours after which the fresh weight has been determined. The oven dry weight is divided over the fresh weight for acquiring the leaf dry matter content.
*Leaf thickness* (mm). Determined with a digital caliper in the middle of the leaf avoiding the main and secondary nerves.
*Specific Force to Punch* (MN m$^{-2}$). The laminas of fresh leaves where perforated with a penetrometer built with a flat-end nail attached to the inner part of a syringe and a waterbasin on top. Water was added to the basin until the leaf ruptures. The total weight

added was converted into Mega Newton and divided over the breaking surface of the hole (circumference of the nail x thickness of the leaf). This alternative punching method shows similar trends compared to cutting and tearing tests and is a good indicator of leaf mechanical resistance (Onoda *et al. submitted*).

*Petiole length* (cm). Determined with a digital caliper or a tape-measure.

*Instantaneous Chlorophyll Fluorescence / $F_0$*. Leaves were dark-adapted for 1 hour after which the fluorescence was measured with a fluorpen (Photon Systems Instruments, FP100) on exposure to light, 455 nm, at a saturating intensity of 3000uE.

*Quantum Yield  ($F_v$ / $F_m$)*. The quantum yield is the ratio between the variable fluorescence and maximum fluorescence on exposure to saturating light (455 nm, intensity of 3000uE) after dark adaptation (1 hour). It reflects the proportion of light used in photochemistry or emitted as heat. Measurements were taken with a fluorpen (Photon Systems Instruments, FP100).

*Wood Density (g $cm^{-3}$)*. With an increment borer (12" mm Suunto, Finland) a core of wood was extracted up to at least the heart of the tree (firmly attached bark or phloem tissue was considered part of the stem). For some species the stems did not reach sufficient size for this method and alternatively a slice of the stem was extracted. The wood cores were divided in three parts (heart, inner wood and outer wood) and the wood density is measured for these parts separately by dividing the dry weight over the fresh volume (where the volume was determined with the water replacement method). These three parts are averaged for the species wood density. This measurement was taken for 32 of the 82 species studied, data on wood density for remaining species were taken from comparable studies in Mexican wet forests in Chajul (Chiapas), Las Margeritas (Puebla) and Los Tuxlas (Veracruz) (Martínez-Ramos & Bongers *unpublished results*).


*Functional diversity*

Functional traits are averaged per species. The abundance of each species in each of the sites is calculated based on the relative number of stems and the relative basal area (DBH > 1cm).

It is calculated as: ($BA_i/BA_{total} + D_i/D_{total}$)/ 2. Where $BA_i$ is the basal area of the $i^{th}$ species, $D_i$ is the density of the $i^{th}$ species and total reflects the total of individuals in the plot. Functional diversity based on presence/absence of species has all abundances equalized at 1. Weighting with this combined measure of density and basal area was chosen because only basal area would bias towards individuals that have the potential to grow a large basal area and we also consider species successful when many individuals are present.

Functional diversity of each of the sites is calculated using the fDiversity software program based on Euclidean distance and average linkage (Casanoves *et al.* 2008). We used the measure of multiplicative extended functional diversity is used (Pla *pers. comm.)*. This diversity measure is based on a dissimilarity matrix of trait-values for all species in the study (Petchey & Gaston 2006) and the matrix is loaded by the relative abundances for each species in that community. Subsequently, the multiplicative extended weighted functional diversity is computed as the total branch length of the functional dendrogram of all species in the community and multiplied by the squared species number. This measure is chosen because it allows including species abundances as (wFD) well as presence/absence of functional traits, when all abundances are set at 1 (FD).

*Statistical analysis*

For determining the relative role of dominant and less-abundant species we calculated a ratio reflecting the functional dissimilarity of dominant species, relative to the functional diversity of the overall community. It is calculated as: ln(wFD/FD). At ratios higher than 0 weighted functional diversity exceeds non-weighted functional diversity, indicating there is selection for dominant species to be less functionally similar, hence an indication for competitive exclusion. A ratio below 0 indicates that dominant species are less functionally diverse compared to the overall species diversity, hence an indication of habitat filtering shaping species dominance. This is calculated for all traits combined as well as for the nine traits separately.

The relation between the functional diversity ratio and fallow age, basal area and species number are tested with regression analysis. For a better description of the trends, the bivariate relationships were additionally analyzed using simple (two-parameter) linear, logarithmic and exponential models. Statistical analysis is carried out with SPSS 17.0.

**Results**

The ln(wFD/FD) ratio, reflecting the relative functional dissimilarity of dominant species (measured over all traits), averaged -0.02 and ranged between -1.2 and 0.9. Our successional factors age and stand basal area did not affect the ratio(R= -.31 p=.359 and R= .44 p=.172 respectively), but species number did (Logarithmic relation, $R^2$ = .38 p= .045) (Figure 3).
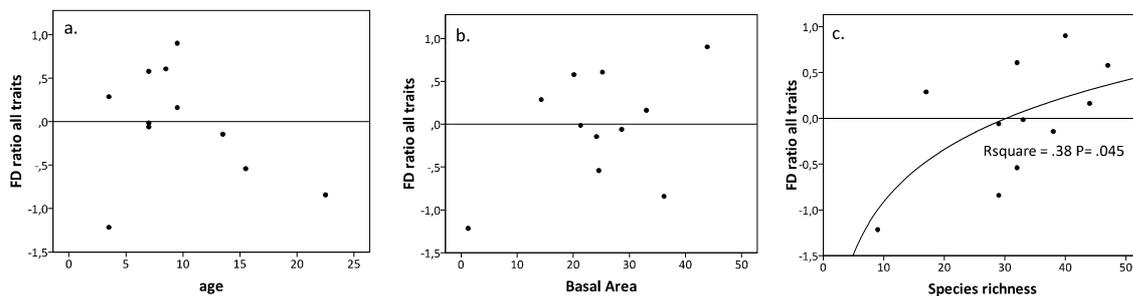


*Figure 3: The wFD/FD ratio (relative dissimilarity of dominant species) for all traits together against successional time reflected indifferent variables. a. ln(wFD/FD) ratio against fallow age of the site (R= -.31; p=.359) b. wFD/FD ratio against stand basal area (R= .44; p=.172) c. ln(wFD/FD) ratio against species number (R= .61; p=.049).*

Because the trend with species number was significant, we plotted the functional diversity ratio's separately for the different traits against species number. Here we found that different traits responded differently in their relative dissimilarity among dominant species (Figure 4). For six out of the nine traits the trait diversity ratio's showed significant positive relations with species number (LA, SLA, LDMC, sFtP, QY and WD). With curve fitting analysis we identified the best descriptor of the data. The functional diversity ratio of LA increased logarithmically with species richness ($R^2$ = .37 p= .046), SLA increased logarithmically with species richness ($R^2$ = .83 p= .000) (Figure 4b), the functional diversity ratio of LDMC increased exponentially with species diversity ($R^2$ = .68 p= .002) (Figure 4c), the functional diversity ratio of specific force to punch increased linearly with species richness ($R^2$ = .40 p= .036) (Figure 4e), the functional diversity ratio of quantum yield increased logarithmically with species richness ($R^2$ = .63 p= .004) (Figure 4f) and the functional diversity ratio of wood

density increased logarithmically with species richness ($R^2$ = .58 p= .006) (Figure 4h). The positive relationships indicated that as species number increases, dominant species are increasingly selected to be functionally dissimilar for the particular traits to allow species to coexist. Following deterministic theory on community assembly, this indicates that there is an increasing role of competitive exclusion and a decreasing role of habitat filtering, as the number of species increases.
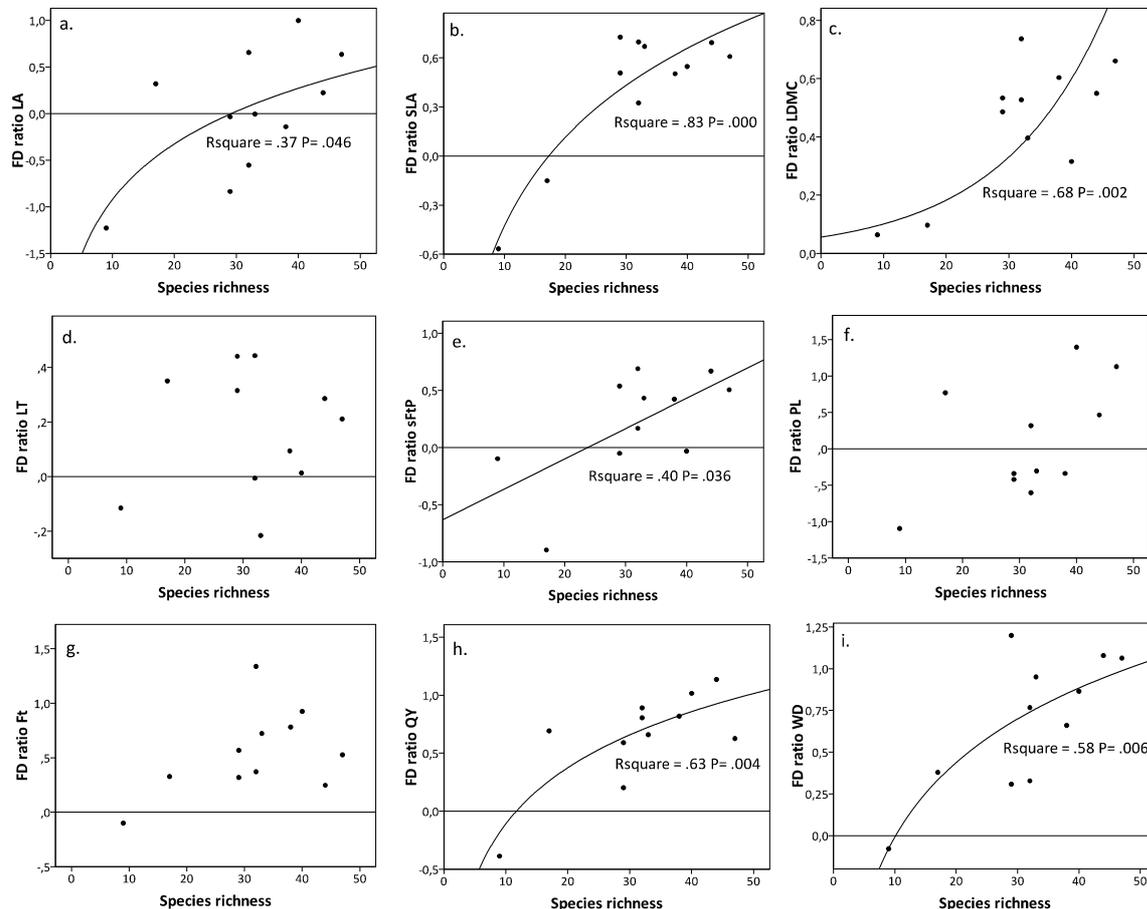


*Figure 4: The ln(wFD/FD) ratios (relative dissimilarity of dominant species) for the separate traits. a. Leaf area diversity ratio against species number (R= .61; p=.048), b. Specific leaf area diversity ratio against species number (R= .83; p=.002), c. Leaf dry matter content diversity ratio against species number (R= .74; p=.009), d. Leaf thickness diversity ratio against species number (R= .10; p=.771), e. specific force to punch diversity ratio against species number (R= .63; p=.036), f. Petiole length diversity ratio against species number(R= .54; p=.083), g. Instantaneous chlorophyll fluorescence diversity ratio against species number (R= .45; p=.162), h. Quantum yield diversity ratio against species number (R= .73; p=.011), i. Wood density diversity ratio against species number (R= .75; p=.008).*

## Discussion

Results suggest that in the secondary forest sites the dominant species are not a random sample of the species that are present. In line with deterministic models, a shift is observed from dominant species being functionally less diverse than all species present towards dominant species being more diverse than all species present. This can be explained by a

shift from habitat filtering to competitive exclusion since habitat filtering selects for certain functional characteristics that are well adapted to the specific conditions of the site whereas competitive exclusion causes dominant species to complement in functional traits to allow coexistence (*e.g.* Webb *et al.* 2002).

No relation was found between functional diversity ratio of all traits together and fallow time or basal area (Figure 3). A significant logarithmic increase was found with species number (Figure 3c). The fact that species number did explain the trend in functional diversity ratio's whereas stand basal area did not, may indicate that presence of different species is a more important factor promoting competition than simply the presence of biomass, regardless of the species it embodies. This suggests that interspecific competition is more likely to generate the functional divergence among co-occurring species than intraspecific competition is.

The positive relationship with species number is in line with the hypothesis and is explained by an increasing pressure on dominant species to be more functionally dissimilar as the secondary forests host more species. Our results suggests that with species richness a shift takes place in the assembly process where competitive exclusion replaces habitat filtering of functional traits. At early stages of succession with low species richness, few functional profiles dominate (ln(wFD/FD) < 0), signifying functional attraction among dominant species and indicates that habitat filtering is a major force shaping community assembly (Webb *et al.* 2002; Stubbs & Wilson 2004; Cornwell *et al.* 2006; Cornwell & Ackerly 2009). At later successional stages where species richness has increased, the dominating functional traits are increasingly complementary and indicate selection through functional repulsion (ln(wFD/FD) > 0), illustrating that competitive exclusion determines which of the species present are functionally suitable to become dominant (*e.g.* Webb *et al.* 2002; Kraft *et al.* 2008).

The functional diversity ratio shows directional change along a gradient of species number which is consistent with the deterministic models of community assembly. This does however not mean that we reject the existence of neutral theory for the study sites. Neutral theory also predicts a decay in community similarity over time, though not resulting from niche adaptation but explained by the chance effect of immigration from the metacommunity and extinction (Hubbell 2008). Since we did not analyse dispersal limitation and species pool, we can not evaluate whether or not neutral theory applies to our findings. However, due to the directional changes in functional dissimilarity of dominant species compared to the overall community along a gradient of species number, we consider the neutral drift prediction less likely. Instead we explain these changes as representing deterministic community assembly theory.

Kraft *et al.* (2008) also found evidence for occurrence of the two contrasting assembly processes based on a study in tropical forest dynamics plots in Ecuador. First they found that mean trait values (presence/absence) show more variation among quadrants (20 x 20) than within quadrants, which is taken as an indicator of habitat filtering. Then they illustrate that the traits measured are more evenly distributed than predicted from a randomly generated community (based on species abundances) from which niche differentiation (competitive exclusion) can be concluded. Although the methodology differs from what is done in the present study, both studies find results indicating the existence of both contrasting forces in the communities studied.

Previous work in the same region (Chapter 1) showed that environmental filtering is the major assembly force shaping sapling communities, as concluded from the explicatory power

of environmental conditions in differing trait composition among sites. The present study however evaluated all individuals (DBH > 1cm) instead of just the sapling stage. The ontogenetic stage of the individuals studied may provide an explanation for the differences in assembly processes found. Phylogenetic overdispersion increases with age (stem size) (Swenson *et al.* 2007); with tree growth biotic interactions (competitive exclusion) play an increasingly important role.

Cornwell *et al.* (2006) found that Mediterranean-type forests are functionally more similar than expected from a random community and they concluded that habitat filtering shaped these communities. Besides the difference in biome, an important difference between Cornwell's and our study is the difference in selection level (Figure 1). Their research focused on the assembly processes between the species pool and the species found in the community (presence/ absence) by comparing the convex hull trait-space (Preparata & Shamos 1985) of randomly generated communities with the observed communities. Our study, in contrast, emphasized the selection of functional traits of species that become dominant compared to the species that are present. These different levels of assembly processes, shaping species presence and shaping species dominance may be distinct processes with different characteristics (Cingolani *et al.* 2007), as also shown in Chapter 1.

Analyzing the same functional diversity ratio (ln(wFD/FD)) for the different traits separately we find that traits differ in how the relative functional dissimilarity of dominant species develops with species richness (Figure 4). Most traits (6 out of 9) illustrate a directional increase in the relative dissimilarity of dominant species. Few traits (LT, PL, $F_0$) appear not to play a role in determining species dominance along a gradient of secondary succession: where dominant species have similar diversity of trait values compared to the overall community, or at least show no pattern with changing species number. The traits LA, SLA, LDMC, sFtP, QY, and WD clearly increase in functional dissimilarity of dominants along a gradient of increasing species number in the secondary forests where all except LDMC also indicate a shift from dominant species being functionally more similar ((ln(wFD/FD) < 0) to dominants being functionally more dissimilar (ln(wFD/FD) > 0). This indicates that the ecological force shaping the observed community changed from habitat filtering to competitive exclusion. The threshold where the competitive exclusion replaces habitat filtering depends on the trait of interest and lies somewhere between 10-30 species in the 0.05 ha plot, but for LDMC dominant species are more functionally diverse than all species present at all stages of species richness (Figure 4c). Also the shape of the functional diversity ratio trend depends on the trait where functional diversity ratios of LA (Figure 4a) and SLA (Figure 4b) show a logarithmic increase, leaf dry matter content increases exponentially (Figure 4c), specific force to punch increases linearly (Figure 4e) and quantum yield (Figure 4h) and wood density (Figure 4i) show a logarithmic increases.

Our hypothesis of shifting importance of assembly processes is based on a model of limited resources. When all niches are occupied, competition limits further colonization of functionally similar species and instead starts favoring functionally dissimilar species through competitive exclusion. The distinct trends shown by different traits suggests that traits are selected by different abiotic conditions which can become limited at different stages of succession. For example, the leaf traits LA and SLA may be limited by light availability, and light may become limited at 20-30 species per 0.5 ha plot (Figure 4a and b). On the contrary, quantum yield and wood density seem to shift at a lower species richness of about 10 species. These traits may be limited by competition for a resource other than light that

apparently reaches a limiting state somewhat earlier in succession. Addressing the wFD/FD ratio of LDMC is especially interesting because at all times this ratio is positive indicating that dominant species are more functionally diverse compared to all species present, and the relationship increases exponentially. This may indicate that the resource selecting LDMC is at all times limited and becomes (exponentially-) increasingly limited as more species enter the site.

The shift along a gradient of species number does not necessarily translate to a shift that occurs with time since abandonment but since all sites were completely cleared of original vegetation at the start of succession it is likely that sooner or later each site will be characterized by a change in assembly process as the number of species in a site accumulates.

**Conclusion**

Comparing functional diversity including species abundances with functional diversity based on presence/absence, conclusions can be drawn on what ecological forces have selected some of the species to become dominant based on their functional characteristics.

We found that indeed dominant species are selected from the species present based on their functional traits through contrasting assembly processes. Our results indicate that already in early stages of succession assembly processes shift from habitat filtering (functional attraction of dominants) to competitive exclusion (functional repulsion of dominants) along a gradient of species number. Moreover, traits show distinct patterns in their relative dissimilarity of dominant species suggesting that traits are selected by specific conditions that reach a limited state at different stages of succession.

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