Linkages between leaf traits and productivity in two resource-limited ecosystems

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Declaration

I declare that this thesis has been composed by myself and has been submitted for any other degree. The work described is my own, except where otherwise indicated.

Cristina Chinchilla Soto

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Abstract

Leaf traits have long been used to classify and characterise species in natural ecosystems. In addition, leaf traits provide important information about plants' strategies for the use of resources and can be used to improve our understanding of ecosystem level processes such as nutrient cycling and carbon allocation. To explore the linkages between leaf traits and productivity, we worked in two resource-limited ecosystems (a grassland and a forest), and used leaf traits to understand how species respond to changes in available resources and their relationship to ecosystem processes. We worked in a species rich limestone-grassland located in central England, which has been subjected to long-term climatic manipulation (winter warming, summer drought and extra summer rainfall). We characterised species composition in terms of their identity, abundance and leaf structural properties (nitrogen content and leaf mass per area (LMA)) in the main treatments and the control. We found that change in species abundance was the most important factor to understand the differences in productivity (above ground biomass and total foliar nitrogen). We then measured CO_2 exchange at ecosystem level, using a chamber technique, and assessed the treatments' effect on the gross primary productivity (GPP) and ecosystem respiration (R_{eco}). GPP and R_{eco} were controlled by soil moisture and above ground biomass but also influenced by the conditions experienced during the growing season prior to the measuring period. Our second location was a post-disturbance chronosequence in a seasonally dry tropical forest in Costa Rica and we used leaf level gas exchange measurements to explore the role of nitrogen (N) and phosphorus (P) on the temporal-spatial variation of photosynthesis of dominant species. We found that photosynthetic efficiency was strongly linked to leaf N and P content, but that there was an important seasonal pattern on this relationship likely associated to P remobilization. Additionally we found seasonal changes in resources (water, nutrients) had a larger impact on the photosynthetic parameters than changes along the chronosequence. The two ecosystems studied for this thesis are contrasting in their physiognomy, species composition and climate, but are also characterised by species whose structural traits (high LMA and high C:N ratio) are likely to have a significant impact on the nutrient cycling processes. We

learned that leaf traits provide important information about species strategies and their usage of resources and they can also aid to address questions at ecosystem level in time and space, either through simple aggregation or as emergent properties. Additionally, the traits explored are important input information to up-scale processes from leaf to the ecosystem level, a step needed to address the effect changes in resources will have on the seasonally dry tropical forest and grasslands, which represent a significant fraction of the total global carbon storage.

“Aprender a leer es lo más importante
que me ha pasado en la vida".

Mario Vargas Llosa,
Nobel Prize Award Ceremony
December 2010

Table of Contents

Chapter 1. Introduction

Introduction

Plants' carbon acquisition depends on their ability to use available resources and to respond or adapt to changes in their supply. Thus, at the ecosystem level the combination of species with different strategies for the foraging of light and nutrients is essential to maximizing the use of resources (Powers and Tiffin, 2010, Reich, 1995), particularly in resource-limited ecosystems (Huston, 1980). This not only might determine ecosystem productivity, but it also has a direct impact on nutrient cycling and the modification of processes at soil level at different temporal and spatial scales (Bardgett *et al.*, 2013).

Understanding how species and ecosystems respond to changes in available resources, primarily water and nitrogen, has long been the research focus of ecology and agriculture. However, since the effect of human activity on global biogeochemistry (increment in CO_2) has become more evident (Schulze, 2006), new research questions have emerged; especially related to the tropical forest, which represents approximately 34% of the total global gross primary productivity (Beer *et al.*, 2010), and could be greatly impacted under current climatic predictions (Cox *et al.*, 2013).

This need to improve our understanding about the controlling factors defining ecosystem productivity, and how these drivers might influence processes across different levels of ecological organization (Chapin III *et al.*, 2009, Suding *et al.*, 2008) has intensified the use of research approaches ranging from leaf to plant as well as to ecosystem level. These allow us to understand how the new stresses might alter the ecosystem's carbon storage in natural and semi-natural ecosystems and possible feedbacks into the climate system.

Three interconnected approaches (long-term monitoring studies, field-scale manipulations and ecosystem processes modeling), have mainly been used to assess climatic effects on the ecosystem productivity in natural and semi-natural ecosystems. Each approach has advantages and constraints. Additionally, although the extension and geographical area covered is not yet balanced, they have shown that ecosystems responses are neither simple nor unidirectional, since ecosystems are rarely limited by a single factor (Hedin *et al.*, 2009).

The first approach involves the repetitive measurement of individual components of the ecosystem over an extended period of time. This has been the predominant method to assess changes in forest productivity, combined with the use of micrometeorological and gas exchange techniques (Malhi, 2012). Although long-term studies are labor-intensive, particularly in forests (Clark and Clark, 1999), they are critical in detecting and understanding an ecosystem's responses to climate (Phillips *et al.*, 2002). Moreover, they have contributed to exploring the response to inter-annual variability in weather conditions (Enquist and Enquist, 2011), and the multiple controls that would determine ecosystem response to CO_2 increments (Clark *et al.*, 2013, Beedlow *et al.*, 2004).

Over the last couple of decades, an important number of field-scale manipulations have explored the effect of changes in temperature and precipitation associated with raising atmospheric CO_2. These experiments suggest that the response will depend on the studied ecosystem, its dominant limitation (i.e. water, nitrogen, light), and the type of manipulation imposed (Beier *et al.*, 2012). Additionally, these experiments have shown that the treatment effect can be significant during the first few years (i.e. changes in species composition seen by Milbau *et al.* (2005) and Grime *et al.* (2008)), but that the ecosystem stabilizes later (Fu *et al.*, 2009, Wu *et al.*, 2011). Clearly, the effect of the manipulations can also be influenced by changes in fertility (Bardgett *et al.*, 2013, Shaver *et al.*, 2006) and the effect of other atmospheric factors (i.e. nitrogen deposition (Phoenix *et al.*, 2012)). Although, most of the examples from field-scale manipulation are originated from grasslands and short stature ecosystems, as large scale manipulations are challenging, some successful examples of large-scale forest manipulation exist (Fisher *et al.*, 2006, Sheppard *et al.*, 2008).

The third relevant approach involves the development and use of models that simulate a variety of plant and soil physiological processes, and their response to changes in climatic conditions and available resources. These models, working at different spatial scales and at different levels of ecological and biological processes detail (Ostle *et al.*, 2009), have produced some important global predictions, in terms of carbon losses and gains (Beer *et al.*, 2010, Huntingford *et al.*, 2013) under the forecasted climate change scenarios (IPCC, 2007). Yet it is clear that these predictions depend on the input data (with some areas still largely underrepresented (Domingues *et al.*, 2010)), the internal assumptions for each cycle, how the data are aggregated from leaf to canopy or ecosystem level (Meyer *et al.*, 2010, Clark *et al.*, 2011), and the level of model detail needed (Meir and Pennington, 2011).

Despite the complexity and the spatial-temporal variability of ecosystems' responses to environmental drivers, it is clear that the combination of field-scale manipulation experiments and species or ecosystem level studies is important to generate knowledge about ecosystem processes (Bier 2004). Furthermore, the up-scaling of these results also allow to assess possible responses to new scenarios. In this context, we have used a limestone-grassland and a seasonally dry tropical forest to understand, not only how leaf traits can reflect a plant's responses to prevailing conditions under limited resources, but also how these traits can be linked to ecosystem responses under changing climatic conditions. Although the two ecosystems are different in their physiognomy, species composition and climate; they are both characterized by the existence of pulses of resources (Bullock *et al.*, 1995, Fridley *et al.*, 2011), by phosphorus limitation (Grime and Curtis, 1976, Vitousek *et al.*, 2010) and by a high species diversity (Leiva *et al.*, 2009a, Grime *et al.*, 2000). This allows for the exploration of concepts and ideas that apply to both.

Thesis rationale: leaf traits as a tool to understand plant responses and to predict community/ecosystem responses

The use of leaf traits to characterize the use of resources in ecosystem and classify plants into productivity groups has a long history, defining some of major axes of modern flora variation (Grime *et al.*, 2007, Craine, 2009). Leaf traits have a demonstrable link to the organism function (de Bello *et al.*, 2010) and potentially significant influence on its establishment, survival, and fitness, as well as on their ability to utilize resources (Reich *et al.*, 2003). This heavily influences the way organisms interact with the physical environment and affect ecosystems processes (Chapin III *et al.*, 2002). Leaf traits also provide a means to compare species, explain species distribution along gradients, and demonstrate more complicated interactions (Ackerly and Cornwell, 2007, Quesada *et al.*, 2009, Givnish, 1988, Westoby and Wright, 2006).

Among leaf traits, the leaf nitrogen content, leaf age and specific leaf area (SLA, ratio of leaf area to leaf dry weight) or its inverse (LMA, leaf mass per area) are the most studied ones. Because they are directly related to the overall plant performance (Westoby and Wright, 2006) and also because they are easy to obtain and can be linked to more complicated or less accessible traits related to biomass production (Delagrange *et al.*, 2008, Reich *et al.*, 2007). Moreover, a large number of studies have suggested classifying species, particularly for up-scaling purposes, considering these set of functional traits instead of using a priori defined functional types or taxonomic approaches (Cornelissen *et al.*, 2003, Powers and Tiffin, 2010, Violle *et al.*, 2007).

Thus far the combination of multiple data sets on leaf traits across the globe (Reich *et al.*, 2007, Kattge *et al.*, 2011), has enabled exploration of general relationships between structural investment (LMA), photosynthetic rate, leaf life span and nitrogen content. The intense study of traits across biomes has originated the idea that most of the variability on leaf traits can be expressed by a single axis of variation (LES, Leaf economic spectrum, (Wright *et al.*, 2004)). This axis essentially represents differences across species in the cost of investing in a unit of leaf area and in the duration of the revenue stream that arises from the investment (Westoby *et al.*, 2013). It is also related to the existence of a trade-off between allocation to structural tissues versus liquid phase processes (Shipley *et al.*, 2006).

Despite the large number of publications on leaf traits across ecosystems, it is clear that the relationships stated in the LES might decouple, importantly impacting up-scaling purposes. This decoupling is likely in ecosystems where the existence of species with a unusual trait combination (Reich *et al.*, 2007), perhaps to allow resistance in resource-limited environments, partially decouples leaf life span and LMA, as was observed by Wright *et al.* (2004) in their original study. This is central for ecosystems dominated by strong seasonality (Gotsch *et al.*, 2010, Domingues *et al.*, 2010, Brodribb and Holbrook, 2005), where additional factors like the high intra-specific trait variability (Hulshof and Swenson, 2010, Tomlinson *et al.*, 2013) play a contributing role.

Leaf traits can be linked to ecosystem level properties in at least three ways that are relevant for this thesis. First, through simple weighted aggregation that takes into account the contribution of each individual to the overall value of the community. This approach considers the number, the relative abundance (biomass) and the identity (through the traits selected) of the species (Garnier *et al.*, 2004, Grime, 1998), providing an idea of the effect of functional diversity on ecosystem functioning (Leps *et al.*, 2006). Secondly, leaf traits can be explored as the property of an entire system (emergent properties *sensu* Salt (1979)). Two commonly used emergent properties, total foliar nitrogen (g of N m^{-2} ground area) and leaf area index (m^2 leaf area m^{-2} ground area), have been shown to be linked to gross primary productivity (Williams and Rastetter, 1999, van Wijk *et al.*, 2005, Street *et al.*, 2012), making them useful to scale from leaf canopy/ecosystem. Thirdly, leaf traits can be used in mathematical models to predict processes at fine or terrestrial scale (Ostle *et al.*, 2009). These models, discussed earlier, rely mostly on the connection between water availability, nutrients and their influence on photosynthesis, therefore relying strongly on the availability and quality on the input data (i.e. leaf traits).

Although other links between leaf traits and ecosystem functioning could possibly be suggested, by studying leaf traits at these three different levels, we can improve our understanding of the strategies that plants use to respond to current and long term environmental stress. For this purpose we selected two resource-limited ecosystems (a grassland and a dry forest), were the richness of species provides the possibility to addressing multispecies responses to changes in resources. Additionally both ecosystems represent a significant fraction of the total global carbon storage pool so understanding the role of the main drivers of carbon allocation processes has important implications to enforce conservation strategies.

Thesis objective

The main objective of this work is to improve our understanding of the implications that the co-existence of species with different traits for the use of resources has over ecosystem productivity and nutrient cycling. Additionally, we aim to identify how these traits can be linked to ecosystems' responses to climatic factors.

The main questions addressed in this thesis:

-What are the linkages between leaf traits and community functioning/productivity in a grassland subjected to artificial climate change?

- How is gross primary productivity and ecosystem respiration of a limestone grassland affected by imposed climatic manipulations?

- What are the main controls of the temporal/spatial variation in photosynthetic efficiency of dominant species in a seasonally dry tropical forest?

Sites overview and relevance

The limestone grasslands

Grasslands represent approximately 10% of the total carbon storage of the biosphere (Jones and Donnelly, 2004), but as they cover a large area of land surface (approximately 30%), changes due to climate change could have an important impact on the carbon cycle (Risch and Frank, 2007). In temperate grassland ecosystems, there is an important allocation to the belowground components (below:above ground ratio between 2.8 and 4), and 75–80% of the root biomass is in the top 30 cm of the soil, what explains their relevance for carbon sequestration (IPCC, 2006). In the United Kingdom, limestone grasslands cover approximately 40000 ha and support a high biodiversity, with some scarce and rare species. This makes them highly susceptible to over grazing and invasion by non-native species (UKBP, 1998).

Our research site (Figure 1) is a climate change simulation experiment that was established in Buxton, northern England, to identify plant attributes and vegetation characteristics conducive to successful invasion (Burke and Grime, 1996). This grassland has been subjected to variations in winter temperature (+ 3ºC) and alterations in the summer rainfall patterns since 1993. So far only minor sensitivity to changes in summer rainfall patterns have been observed, with no response to winter temperature regime (Grime *et al.*, 2008).

Figure 1. View of the experimental site in the Buxton Climate Change Impacts Laboratory (BCCIL). The photo show the five experimental blocks. *Photo by Louise Beveridge*.

In Buxton, during the summers of 2010 and 2011, we carried out ecosystem-level gas exchange measurements to assess the effects of the climatic manipulation on the gross primary productivity. In 2010 we also characterised species' composition and leaf properties. We then linked these leaf properties, on their own and as emergent properties (TFN and LAI), to ecosystem productivity. Finally, we explored the influence of species composition on the carbon fluxes.

Figure 2. Field activities carried out at BCCIL in June 2010 and June 2011. Clockwise from top left corner: close-up of the gas exchange system, identifying grass species (*photo by L. Beveridge featuring Phil Grime*), measuring biomass properties indirectly (*photo by L. Beveridge featuring Mathew Williams*), sorting grass turfs into species, setting up the system to carry out the light curves (*photo featuring L.Beveridge*).

The seasonally dry tropical forest site

Seasonally dry tropical forests (STDF) are tree-dominated systems (Bullock *et al.*, 1995), characterized by strong rain seasonality which determines the key ecosystem processes (Quesada *et al.*, 2009). Although defining the extension of the SDTF is complex, it has been estimated that the SDTF covers approximately one million square kilometres, with more than half of that area located in the Neotropics (Miles *et al.*, 2006, Linares-Palomino *et al.*, 2011). In combination with savannas (Figure 3), the SDTF extends from Florida in the north of America to Bolivia and Paraguay in the south (Pennington *et al.*, 2006).

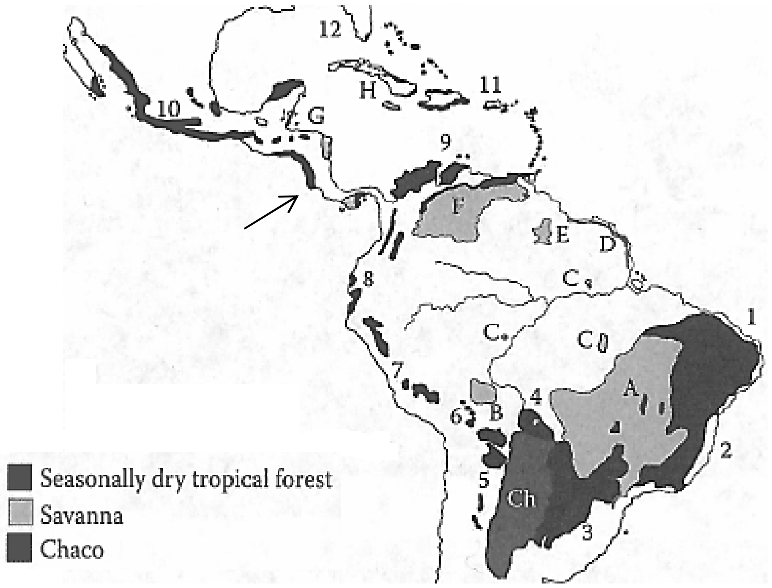

Figure 3. Schematic distribution of the seasonally dry tropical forest (represented by numbers) and savannas (represented by letters) in the Neotropics. SDTF: 1. caatingas; 2. south-east Brazilian seasonal forest; 3. Misiones, 4. Chiquitano, 5. Piedmont, 6. Bolivian inter-Andean valleys; 7. Peruvian and Ecuadorian valleys; 8. Pacific coast Peru and Ecuador; 9. Caribbean coast of Colombia and Venezuela; 10. Mexico and Central America; 11. Caribbean Islands, 12. Florida. Savannas: A) Cerrado, B) Bolivia, C) Amazonia, D) Coastal, E) Rio-Branco, F) Llanos, G) Mexico and Central America, H) Cuba, Ch: Chaco (not SDTF or savanna). From Pennington *et al.* (2006). The arrow points to the approximate location of the study area.

Net primary productivity for the SDTF, estimated from aboveground biomass inventories has been reported between 8 and 21 (t dry aboveground biomass ha^{-1} y^{-1}) (Murphy and Lugo, 1986, Martinez-Yrizar *et al.*, 1996). This value is lower than the reported for rain forest, mostly due to a lower leaf area index (Kalacska *et al.*, 2005) and to a seasonal decline in assimilation rates (Meir and Pennington, 2011). Species in the SDTF tend to allocate large fractions of photosynthates (up to 50%) to the development of underground biomass (Bullock *et al.*, 1995, Martinez-Yrizar, 1995), necessary for water and nutrient uptake during prolonged stress periods (Holbrook *et al.*, 1995).

The area where we developed our project (Figure 4), is located in the north-western area of Costa Rica and is part of one of the biggest efforts of protection and restoration of the SDTF (Sanchez-Azofeifa *et al.*, 2005, Janzen, 2002) in the Mesoamerican region.

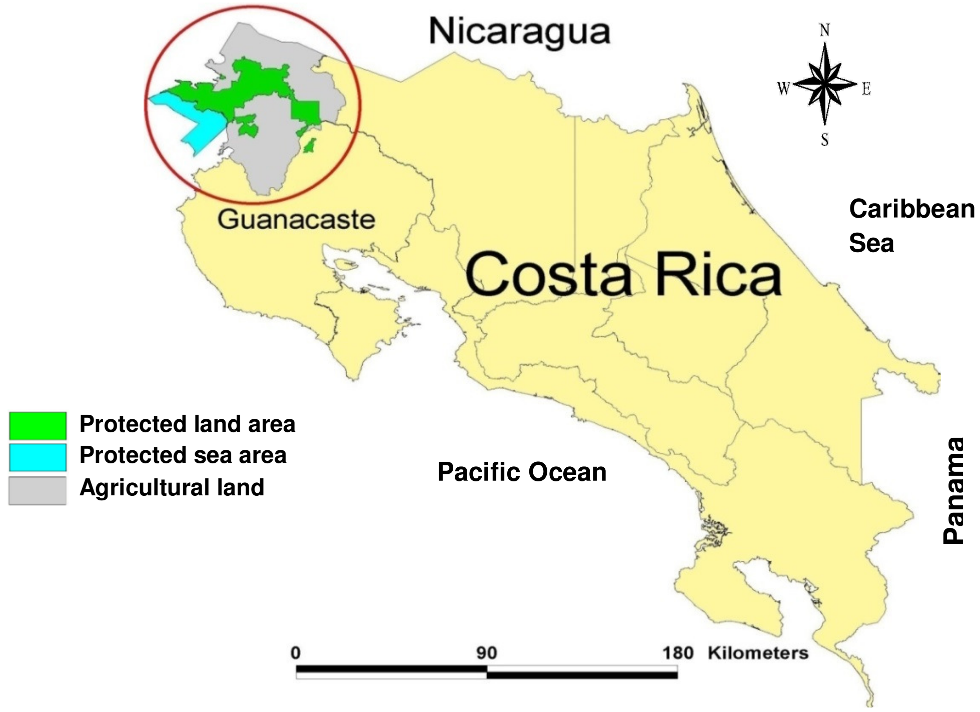

Figure 4. Location of study site (red circle), part of the Guanacaste Conservation Area (ACG) in Costa Rica. The protected land and sea area represents almost 1630 km^2 (and it includes transition into wetter forests). The SDTF represents approximately 10-15% of the total area. Map elaborated by Waldi Medina ACG, November 2003.

For this study, we used a post-disturbance chronosequence (Leiva *et al.*, 2009b) and between May 2011 and January 2012, we measured the seasonal and spatial variation (at plot level) of some of the key ecophysiological parameters (Figure 5) that define productivity (photosynthesis). Additionally, we studied the relationship of the photosynthetic parameters with some commonly measured structural traits (LMA, nitrogen and phosphorus content). As four of the selected species were present across most of the chronosequence, we also explored seasonal/spatial patterns at species level.

Figure 5. Field activities carried out at the seasonal dry forest field site between May 2011 and January 2012. Clockwise from top left corner: measuring diameter at breast height (*featuring Christina Smith and Saul Chévez*), hemispherical photography for leaf area index estimation, collecting the branches from a *Hymenaea courbaril* (*photo featuring Adrian Guadamuz*), phenological observations, measuring CO_2 exchange in leaves, measuring water potential.

Although we only worked at leaf level for this study, our discussion addresses how this information could be up-scaled to the canopy level through process-based modelling (Williams *et al.*, 1996), to address changes in productivity due to seasonality in water availability. This could be relevant to understand the effects of the inter-annual climatic variability created by the El-Niño-Southern Oscillation (Enquist and Enquist, 2011), as well as the possible effects of climate change predicted for the region (Enquist, 2002).

Thesis Structure

This thesis is an amazing journey from the limestone grasslands in the United Kingdom to the seasonally dry tropical forest in Costa Rica. Despite the logistical complications involved in conducting simultaneous research in geographically distant ecosystems, this framework makes generating new hypothesis possible, to compare and contrast strategies in the use of resources and to assess the strengths and limitations that field approaches/techniques have when they are used across both ecosystems.

The thesis is written as individual papers that will be submitted to publication and the main content of each paper is outlined below. For paper 2 and 3 there is a section of supplementary material that contributes to the understanding of the main information presented on the papers. In the final discussion I summarize the main findings and implications of this work, and suggest future research paths.

Paper 1: Implications of leaf traits variation for grassland productivity in a long-term climate manipulation

The objective of this paper was to assess the relevance of leaf traits for community functioning and their response to the imposed climatic treatments. We explored variations in leaf mass per area (LMA) and leaf nitrogen content (LNC) and hypothesised that LMA would vary following predicted patterns of variation along climatic gradients (higher where water is limiting) but that LNC would be limited by nitrogen availability at the site. We found that, at community level, the treatments significantly affected LMA and shifted LNC and the patterns of variation in the LMA-LNC relationship were probably altered by species composition and abundance. Additionally, we explored how the effect of the treatments would affect the relationship between leaf area index (LAI) and total foliar nitrogen (TFN), an important tool to scale from leaf to canopy level. We hypothesized that biomass will drive this relationship but that it will be affected by the treatments. We found that although both parameters were coupled across the experiment, in the drought treatment, the TFN-LAI relationship was controlled by a trade-off between LMA and LAI. The TFN of this ecosystem was higher than values reported for other natural ecosystems. We suggest that given the TFN-LAI relationship slope was smaller than the ecosystems we compared it to, there is a tighter N cycling in the experiment. We conclude arguing that the TFN-LAI relationship could be driven by the dominant species in the community.

Paper 2: Soil moisture, leaf area index and inter-annual climatic variability control gross primary productivity in limestone grasslands in a field-scale climate manipulation experiment

The main objective of this paper was to evaluate how gross primary productivity (GPP) and ecosystem respiration are affected by the imposed climatic manipulations in Buxton. We hypothesized that variations in the fluxes would be tempo-spatially complex and important variations will be driven by differences in soil depth and water limitations (i.e. the productivity would be lower at the drought treatment). Additionally, we hypothesised that, as it has been observed in other ecosystems, where this technique has been used, we would find a strong link between gross primary productivity and above ground biomass (LAI). Finally, we explored the relationship between species composition and the carbon fluxes. Contrary to our expectations, we did not find a direct effect of soil depth in the magnitude of the fluxes, but productivity was certainly lower at the drought treatment. However, the drought treatment was the only one where the expected relationship between biomass and GPP was significant. Additionally, we found that the fluxes were also affected by the growing conditions experienced during the previous season, whose main effect was on the ecosystem respiration. Therefore, we propose that the observed response is the result of an interaction between aboveground biomass and soil moisture, and that soil depth is relevant for rooting depth but not for water availability. We finally suggest that the stability observed in the net primary productivity (biomass harvest at the end of the year) might be explained by a higher allocation to belowground structures in the water limited treatments, and by fast leaf turnover in the watered treatment.

Paper 3: Spatial and seasonal variability of photosynthetic parameters and leaf traits in a Neotropical seasonally dry forest

The objective of this paper was to explore the main controls on the photosynthethic efficiency of dominant species in the SDTF, evaluated on a seasonal scale and along a successional gradient (plots of 15, 25, 65 and 100 years of regeneration). We hypothesized that at plot level the photosynthetic parameters (the maximum rate of carboxylation, V_{cmax}, the electron transport driving regeneration of the ribulose 1.5-bishposphate, J_{max}, and the photosynthesis at saturating light conditions, A_{sat}), will mostly be driven by the changes in leaf nitrogen content but that phosphorus could also be important in the changes in J_{max}. Additionally, we hypothesize that the bivariate relationships between photosynthetic parameters and structural properties would not be affected by the season or the successional stage. We found that the photosynthetic parameters had no major seasonal variation with the exception of $A_{\text{sat.}}$ However A_{sat} was more responsive to changes in stomatal conductance than to changes in nutrient content. In addition, we also found that V_{cmax} and J_{max} are equally controlled by nitrogen and phosphorus, particularly during the rainy season, perhaps related to the existence of phosphorus limitation in this ecosystem. We also explored how photosynthetic capacity of species that were present along the chronosequence, would vary along the successional gradient. However, contrary to our hypothesis, species in the early stages of the chronosequence did not show higher values in their photosynthetic traits. The parameters reported here are new for the site and are relevant for up-scaling purposes.

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Chapter 2. Implications of leaf traits variation for grassland productivity in a long-term climate manipulation

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Abstract

To assess the relevance of leaf traits for community functioning, we explored how climate change has modified leaf mass per area (LMA) and leaf nitrogen content (LNC) in limestone grasslands subjected to drought, supplemented rainfall or winter warming in a long-term climate change experiment in Buxton, United Kingdom. We harvested the aboveground biomass across 30 plots and examined if LMA and LNC at species and community level changed across the treatments following predicted patterns for water limitation. Additionally, we explored the role of the within-species variation on LMA. Our results showed a high variability at leaf level for both LMA (13.7 to 164.4 g m^{-2}) and LNC (7.4 to 31.6 mg N g^{-1}) across the experiment. Between-species variability of LMA dominated the within-community variability (approximately 80%). Additionally, at community level the treatments significantly affected LMA (higher in the drought plot) and shifted LNC at community level (lower in all treatments), mainly through changes in species composition and abundance. Finally, we estimated total foliar nitrogen (TFN) and leaf area index (LAI), critical determinants of gross primary production, and explored how the treatments have affected their relationship and the possible role of leaf trait adjustment on the observed results. We found that the TFN of this ecosystem (1.5 to 6 g N m^{-2}) was higher than values reported for Arctic or prairies ecosystems. However, the slope of the TFN-LAI relationship (0.71 g N m^{-2} LAI) was smaller, suggesting that a tighter N cycling in the experiment and the ability of species to explore different nutrient pools might be essential to maintain productivity.

Introduction

In recent years, there has been an increasing interest in the study of plant traits enhanced by a widespread acceptance of functional traits as controllers or indicators of community performance (Reich *et al.*, 2003, Ackerly and Cornwell, 2007); and by the idea that the type, the range and the relative abundance of functional traits in a community exert a significant control over different ecosystem services (de Bello *et al.*, 2010). Additionally, simultaneous analysis of multiple traits allows one to test specific hypotheses about the relationships of functional traits, functional groups and their performances (Roberts *et al.*, 2010, Gitay and Noble, 1997).

Although the mechanisms controlling trait variation within sets of coexisting plant populations and their role in the functioning of communities and ecosystems has been widely studied, (Grime, 2006), not many studies have examined how traits change under long-term manipulations of environment, and fewer still consider changes as both species and populations (or individuals) change (Ravenscroft *et al.*, 2013). This information is relevant for understanding how specific leaf traits influence productivity at ecosystem level, a critical research focus with relevance from field to globe (Reich *et al.*, 2007)

To assess the relevance of leaf traits for community functioning, we explored for a limestone grassland ecosystem how climate change has modified traits at leaf level and as emergent properties of the community. The study site includes a long-term manipulation experiment, where the ecosystem has been subjected to variations in the rainfall regime during the summer (droughting/irrigation) or to winter-temperature (Grime *et al.*, 2008). So far, only minor shifts in species composition have been observed, with a reduction in biomass in the droughting treatment.

We focused our work on the leaf traits known as functional ecosystem markers, leaf mass per area (LMA) and leaf N content (LNC), for their essential role in functional responses to change (Garnier *et al.*, 2004, Garnier *et al.*, 2007). LMA is a key trait on plant growth (Poorter *et al.*, 2009) that represents the leaf-level cost of light interception and represents the investment associated with a given potential rate of return (i.e. light capture area)(Wright and Westoby, 2002). LNC, on the other hand, is directly linked to productivity through the role that nitrogen-rich compounds play in photosynthesis (Hikosaka, 2003). Additionally, we explored the role of these leaf traits in the ecosystem's acquisition-conservation of resources. For this purpose we used the total foliar N (TFN)-leaf area index (LAI) relationship (the total grams of foliar N per square meter of ground and total amount of leaf area on a square meter, respectively), which has proven to be an useful tool to scale from the leaf to canopy or ecosystem level (Williams and Rastetter, 1999, van Wijk *et al.*, 2005, Street *et al.*, 2012).

Despite the extensive research that has been carried out looking at the effect of environmental conditions on leaf traits variation, it is still not clear to what extent the variation of LMA and LNC and their relationship can be modified by site-specific factors (Wright *et al.*, 2005). In general, studies looking at the variation of LMA and LNC along climatic gradients (Sandel *et al.*, 2010, Wright *et al.*, 2005, van de Weg *et al.*, 2009) report leaves with higher LMA in drier sites (i.e. high mean annual temperature or low rainfall), as more rigid leaves with a smaller transpiring surface are essential to withstand water stress (Poorter *et al.*, 2009). Additionally, leaves of drier sites are also expected to have a higher N content on area basis, associated to a more economic water use for carbon fixation (Wright *et al.*, 2001), that usually would represent a lower LNC on mass basis (due to a higher LMA).

It is expected that the patterns of variation of LMA and LNC on this experiment will be determined to a large extent by the individual traits values (species identity) but also by their abundance (contribution to the total biomass) (Garnier *et al.*, 2004). However, they are likely to be influenced by the site's variations in soil N content at small spatial scales (referred to here as microscale variation) (Fridley *et al.*, 2011, Moser *et al.*, 2011), creating heterogeneous responses and making it hard to disentangle the effect of the treatments from the role of species composition and microtopography.

Therefore, in the context of the climate change experiment, this paper seeks to address the following questions:

1. Are LMA and LNC changing across the treatments? Are these traits following the expected patterns along climatic variations?

   H1.1). We hypothesise that LMA will be higher in sites where the combination of treatment-soil properties create drier conditions (i.e. drought or heated-shallow soil plots). LNC, on the other hand, is limited by soil N availability and the available water for its mineralization, so it will not change significantly across treatments.

   H1.2). Our alternative hypothesis is that both LMA and LNC will follow predicted patterns of variation (i.e. increased LMA, decreased LNC on a mass basis in drier sites) despite nutrient limitations on the site.
2. Is the variation observed in LMA dominated by a change in species composition (interspecific) or by a change in species traits (intraspecific)?

   H2.1). We hypothesise that the variation of leaf traits among treatments is linked to interspecific variation, but that important variation is expected due to intraspecific changes.

   H2.2). Our alternative hypothesis is that leaf traits variation among and within treatments is dominated by intraspecific variation.
3. To what extent is the climatic manipulation affecting emergent ecosystem traits such as LAI and TFN? Can variations in LAI-TFN along the experiment be related to the dominant species and their changes?

   H3.1). We hypothesise that the variation in LAI and TFN is driven by the amount of biomass, so it is linked to the dominant species, their biomass and their traits. We expect LAI and TFN to be tightly coupled along the experiment (van Wijk *et al.*, 2005), but that this relationship will be affected by the treatments where different gains in TFN from an additional investment in LAI are expected (affecting the slope of the relationship).

   H3.2). Our alternative hypothesis is that TFN and LAI will vary along the treatments due to changes in species richness, and as a result across communities TFN will differ for communities with similar LAI.

This study provides a unique opportunity to explore trait shifts with climate and site properties, and their major implications for plant dry mass and nutrient economics at ecosystem scale. The study will identify how productivity is regulated under the selective pressures associated with adaptation to different climatic regimes (Wright *et al.*, 2005, Pakeman *et al.*, 2011). Furthermore, looking at individual traits, their dissimilarity, and how they act in natural assemblages (Flynn *et al.*, 2008, De Boeck *et al.*, 2007, Mouillot *et al.*, 2011), contributes to our understanding of the implications that changes in species composition vs. changes in species properties have in litter fall quality, nutrient cycling at ecosystem level, and productivity and how they might feed-back into the carbon cycle. Our study is novel because we consider how individual leaf traits and each species' relative abundance contribute to shape ecosystem productivity.

Methodology

Field Site

The field site is located near Buxton (UK national grid reference SK055706; latitude 53° 20'N), in northern England. The area is located on Carboniferous Limestone at a mean altitude of 370 m (Grime *et al.*, 2000).Soils (classified as Humic Rankers) are shallow (7-40 cm), loamy, with surface leaching and generally low in available plant nutrients (Bates *et al.*, 2005). The area receives approximately 1200-1300 mm of rainfall per year with a mean annual temperature of 8 ºC (Buckland *et al.*, 1997). Prior to enclosure for the present purpose, the site had been an ancient sheep pasture.

Although the grassland of the region has a complex history it is likely that some areas have supported semi-natural grasslands for at least 100 years and that most of the region's forest had been cleared out by the fifth century (establishment of the Anglo-Saxons) (Lloyd *et al.*, 1971). The site is classified as calcicolous grassland according to the British National Vegetation Classification (NVC), and the area is dominated by *Sesleria albicans-Galium sterneri* grassland (CG9) in combination with *Festuca ovina-Avenula pratensis* grasslands (CG2), typical communities of free-draining but moist calcareous litomorphic soils (Rodwell, 1992). The NVC classification is based on percentage cover data for the site analysed with ComKey2 (a vegetation community analysis tool developed by C. Legg of the University of Edinburgh).

Experimental set up

The main experiment is composed of 5 (10 x 10 m) blocks of treatments on a north-west facing slope of approximately 35°. Each block is subdivided into 9 plots (3 x 3 m) that include one control plot, three spare plots, and five experimental plots. The experimental plots have received over 15 years the following manipulations: supplemented rainfall in the summer (watered treatment), rainfall exclusion in the summer (drought treatment), increase of 3° C in soil temperature during the winter (heated treatment), a combination of incremented winter temperature + intercepted rainfall, and a combination of incremented winter temperature + supplemented rainfall) (Grime *et al.*, 2008). In addition to the climatic manipulations, in October the vegetation is cut to a height of 40-50 mm to simulate annual sheep grazing and all cuttings are deposited offsite.

Sampling strategy

The field campaign was carried out during the summer of 2010 and we only considered the control plots and three of the treatments (drought, watered and heated treatment), a total of four plots per block. Within each treatment, we defined a community as the combination of species present within an area of 0.2 x 0.2 m. Each sampled community was randomly assigned within the 3 x 3 m plot. To minimize disturbance level on the experiments, only one sample was taken from each of the main treatments and three samples were taken from each of the 5 control plots (to achieve 30 samples in total).

To randomly assign the sampling points, we laid out two large wood sticks at right angle to each other to create four sections on the plot. The sampling points were placed using tags numbered from 1 to 4 that were drawn from a hat, working clockwise from the top-left section corner. We first selected the section of the plot and then re-drawn the tags to select the position within the section (each section was subdivided in four parts as well) to allocate the final sampling position.

Above-ground biomass, as near to the soil level, was harvested in each community during July 2010. The turfs were placed into plastic bags and sorted into species in the laboratory as quickly as possible. Dry weight of each component in the turf (leaves, stems, branches, bryophytes and litterfall) was obtained after 2-3 days of oven drying at 60° C.

Traits

Leaf level traits

Prior to drying, leaf area for each individual species was obtained using a flat scanner and images were processed using Image J software (Abramoff *et al.*, 2004). For most of the dominant species within a turf, at least 3 sub-samples were scanned. Each species sample and subsample were dried and weighted. The total leaf area of each species was calculated using the average dry weight/leaf area relationship from the sub-samples.

Leaf mass per area (LMA, g m^{-2}) was estimated for each species as the ratio of dry weight to leaf area. LMA was chosen over specific leaf area (SLA, m^{-2} g) because although both traits represent the leaf-level cost of light interception and are derived from the same physical measurements, LMA scales positively linearly with additional investments in leaf structure, while SLA decreases hyperbolically, which makes it harder to interpret (Poorter *et al.*, 2009).

Leaf nitrogen content (LNC, mg g^{-1}) and leaf carbon content (LCC, mg g^{-1}) (to estimate C:N ratio), were determined for the species representing 85% of the total leaf area in each community, using a Carlo Erba NA 2500 Elemental Analyser (Carlo Erba Instruments, Milan, Italy). For those species at a site that were not analysed for N and C content, the concentration was determined from the N or C content of the same species in another community on the same treatment (a different block or the same plot in the control) following the approach used by Williams and Rastetter (1999). 10-14 species per community were analysed for N and C to comply with this 85% threshold.

Community level traits (LTagg)

We evaluated how leaf traits (LMA, LNC and C:N ratio) changed at the community scale. Traits at community level were aggregated as weighted means (LT_{agg}), thus taking into account the individual contribution of each species trait, their abundance and providing a better estimate of their effect on ecosystem functioning (Grime, 1998, Leps *et al.*, 2006). LT_{agg} was estimated with Equation 1, using relative aboveground biomass as an indicator of each species' relevance in the community, according to Garnier *et al.* (2004):

$$LT_{agg} = \sum_{i=1}^{n} p_i \times trait_i \qquad \text{(Eq. 1)}$$

where p_i is the relative contribution of species *i* to the total foliar biomass of the community, and $trait_i$ is the trait (LNC,LMA or C:N ratio) of the species *i*.

Canopy level traits

Canopy level traits were defined as those emergent properties that describe the community on an area basis (i.e. biomass, leaf area index and total foliar nitrogen). The total above ground biomass ($\text{Biomass}_{\text{TOTAL,}}$ g m^{-2} ground area) was estimated as the sum of the dry weight of leaves, stems and branches (when applicable) for all the species present in the community (excluding bryophytes), expressed on an area basis. The leaves biomass ($\text{Biomass}_{\text{LEAF,}}$ g m^{-2} ground area), was estimated as the sum of the dry weight of all the leaves in the community on an area basis. Litterfall on each sample was estimated as the dry weight of all the remaining material on the sample once the above-ground material was removed (just on the turf's surface and not including roots).

Leaf area index (LAI, m^2 leaf area m^{-2} ground area) was determined as the sum of the individual areas of all species (as determined in the leaf traits section) on a plot, divided by the area of sample (0.2 m x 0.2 m). The total foliar nitrogen (TFN, g N m^{-2} ground area) was obtained by multiplying species N content by its dry weight in the sample and then scaling by the sample area.

Data analysis

All tests were performed using R 2.15.2 (R Core Team 2012). Variations in LAI and TFN, and community level traits (LMA_{agg}, LNC_{agg} and C:N $\text{ratio}_{\text{agg}}$) across the experiment were evaluated using a mixed effect model (nlme package in R) with treatment as the fixed effect and with repetition nested in Block as a random factor (to deal with pseudoreplication in the Control plots). The validity of the mixed effects analysis was checked with likelihood ratio tests comparing the models with fixed effects to the null models with only the random effects (if the model including fixed effects did not differ significantly [$p<0.05$] from the null model the results were rejected). Simple comparisons of means between treatments and control plots (unless otherwise specified) were carried out using the "contrasts" option of R within the mixed effect model analysis.

Standardized major axis slopes regression (SMA) with the smatr package in R (Warton et al., 2012), was used to summarise the bivariate relationship between variables (i.e. the relationship between LMA and LNC at leaf level) and to compare the slopes (quantify the effect of the treatments) (Wright *et al.*, 2005). Previous to this analysis variables were log-transformed to improve normality (Warton *et al.*, 2006). Simple linear regression (OLS) was preferred when the main interest was the predicting-equation between two variables (i.e. TFN and LAI relationship), in this case comparison of slopes was performed via ANCOVA. Slopes are referred throughout the text as β.

Since TFN is the direct product of LAI x LNC and therefore it becomes zero when LAI is zero, for the TFN-LAI relationship and the relationship with leaf biomass the regression line was forced through the origin. Although forcing a regression through the origin is seldom recommended (Quinn and Keough, 2002), the model with the intercept was compared to the model without intercept by comparing the mean square error from the ANOVA table, the F value and the standard error of the predicted slope and the model with the better set of these parameters was chosen (the non-intercept model for the TFN-LAI.)

The extent of the variation of the LMA and LNC along the experiment was evaluated at the different levels (species level across the experiment, within sample [community], within treatment and at the experiment level) by estimating the coefficient of variation (CV). As there was a higher number of intra-species repetitions in each community for LMA than for LNC, we determined the among and within-species contribution to the LMA dissimilarity using the approach described by Leps *et al.* (2006), that uses the variance of species trait values weighted by the species relative abundance. Only between 10 and 12 dominant species per community were used for this analysis (mostly grasses and forbs).

Results

Vegetation composition

The site was characterized by a high diversity of species that ranged between 12-22 species per sample (Table 1), within the range previously described for the site (Grime *et al.*, 2008). The watered treatment was the only one where species richness (number of species in a sample) was significantly different from the control (p<0.01, Simple contrasts in nmle).

No species was dominant in more than half of each individual plot, except for *Helictotrichon pratense* (grass) that was dominant on 30% of the samples on Control plots and *Festuca ovina* that was dominant on half of the samples evaluated at the Heated Treatment. When the species were grouped by functional type (grass, sedge, shrub and forbs), the samples were in general dominated by grasses (34-48% abundance), followed by forbs (28-37% abundance), except for the watered plots that had a more homogeneous composition in terms of functional groups (excluding shrubs) (Table 1).

Table 1. Treatments' functional-group composition. The data is the average of the % of dominance of each functional group at each treatment ± standard deviation and therefore it might not add up to 100% exactly. The composition was estimated from the abundance of each species in terms of biomass (n=15 for control plots and n=5 for treatments). Species richness refers to the number of different species present in a sample.

Treatment	Composition (%)												Species Richness		
	Grass			Sedge			Shrub			Forbs					
Control	46	±	14	18	±	9	8	±	14	28	±	11	17	±	1
Drought	42	±	12	19	±	6	1	±	2	39	±	14	18	±	1
Heated	48	±	11	20	±	4	0	±	0	32	±	9	19	±	1
Watered	35	±	15	31	±	10	0.2	±	0.4	34	±	16	21*	±	1

*significantly different from the control (p<0.01 simple contrasts in nlme).

Leaf level traits

Leaf mass per area (LMA) and Leaf nitrogen content (LNC)

Values of LMA at species level ranged from 13.7-164.4 g m^{-2} (mean 49.5 ± 20.6 g m^{-2} [standard deviation]), across the experiment and 7.4-31.6 mg N g^{-1} for LNC (mean 14.7 ± 4.7 mg N g^{-1}). At species level, there was a significant inverse correlation between LMA and LNC ($r = -0.24$, $p<0.001$, $n=199$). Although the correlation for LMA and LNC at leaf level was significant across the experiment (when the entire data set was considered), the high natural variability at the site precluded the finding of a significant correlation where traits were grouped by treatment except for a weak relationship at the Watered ($r=-0.28$, $p<0.1$, $n=44$) and the Heated Treatment ($r=-0.30$, $p<0.1$, $n=39$). However, the general pattern of LMA and LNC being inversely correlated at treatment level prevailed (Figure 1). When only the most abundant species (Table S 1) were considered, the LMA presented a larger coefficient of variation (42%, n=512) than the coefficient of variation for LNC (32%, n=188).

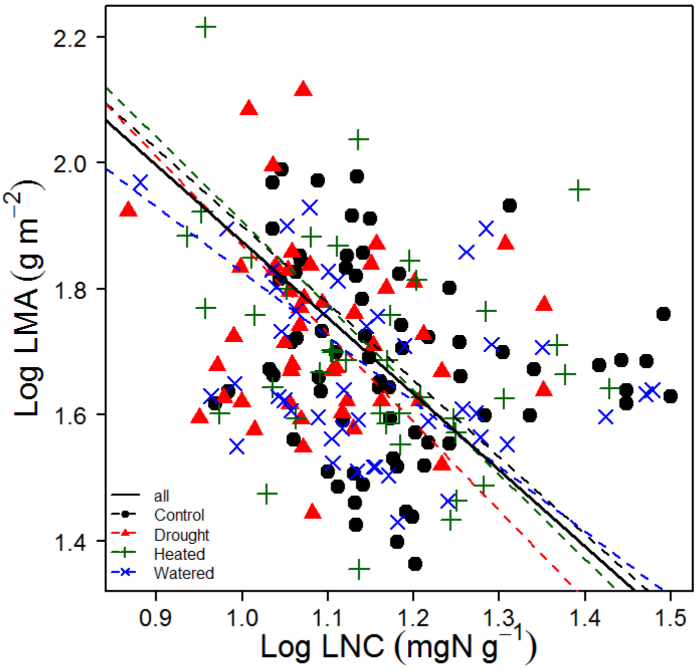

Figure 1. Relationship between leaf mass per area (LMA) and leaf nitrogen content (LNC) and LNC, partitioned by treatment. Linear regression lines were fitted with SMA and are shown for illustrative purposes even when not statistically significant. **Control**: β=-1.22, R^2=0.03, p=0.18, n=69. **Drought Treatment:** β =-1.41, R^2=0.02, p=0.40, n=47. **Heated Treatment**: β =-1.34, R^2=0.1, p<0.01, n=39, **Watered Treatment**: β=-1.031, R^2=0.08, p<0.01, n=44, **All:** β =-1.21, Intercept=3.089 R^2=0.1, p=<0.001, n=199. Each point represents an individual species. Slopes were not compared as the correlation between traits was not significant for the control and heated treatment. Untransformed values of LMA varied from 13.7-164.4 g m^{-2} and 7.4-31.6 mg N g^{-1} for LNC.

Although the LMA-LNC relationship was not significant at treatment level, it was significant when examined considering the dominant functional types (Figure 2) and significant differences between slopes were observed (Likelihood: 38.74, DF=3, p<0.001). In the regression line for the Forbs a change in the slope (from -0.84 to -1.04) was observed when the legumes were excluded from the analysis, however the two slopes were not significantly different (Likelihood ratio statistic: 1.57, DF=1, p=0.21).

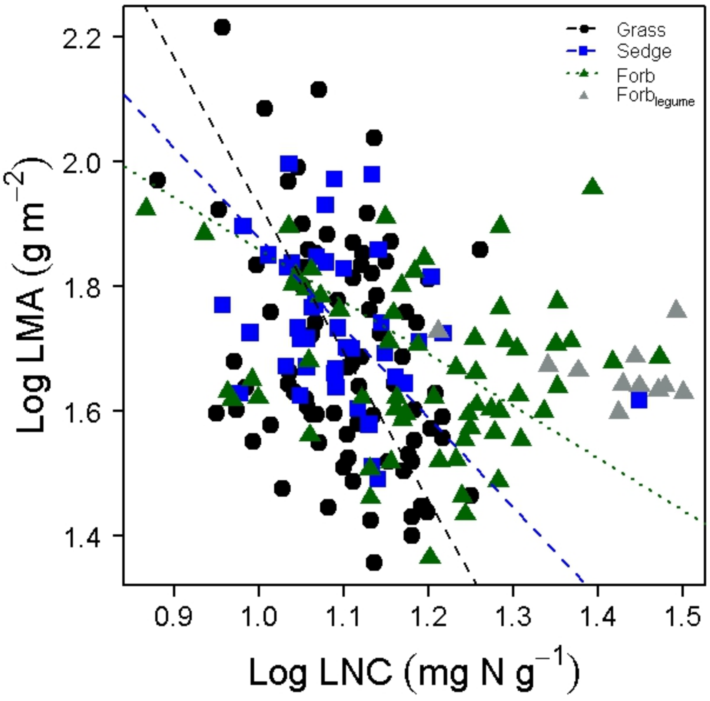

Figure 2. Relationship between leaf mass per area (LMA) and leaf nitrogen content (LNC), partitioned by functional type. Linear regression lines were fitted with SMA and were significant or marginally significant except for shrubs (not shown) **Grass**: β= -2.40, R^2=0.1, p<0.01, n=78. **Forb:** β =-0.84, R^2=0.04, p<0. 1, n=66. **Sedge**= β =-1.44, R^2=0.1, p<0.1, n=43. Each point represents an individual species. The regression line for the forbs includes the legumes (represented with a different colour in the figure). Slopes were significantly different among functional types.

The LMA-LNC relationship was further examined considering soil depth classes (0-7, 8-12, and 13-20 cm), and was significant for sampling undertaken in sites with soils in the deepest class (R^2=0.2, p<0.05). Species in the drought plot showed the strongest positive correlation between LNC and soil depth (r=0.37, p<0.05, n=47), and species in the watered plot showed the strongest correlation between LMA and soil depth (r=0.25, p<0.05, n=44).

Within sample variability of LMA

The within-sample variability (the coefficient of variation of the trait within each community) was similar across the experiment (Table 2), although higher in the heated treatment. When the contribution of the between-species variability and the within-species variability (how much the trait varied within a species) was considered for LMA (as the number of within species repetitions precluded this analysis for LNC), in general the contribution of between species variability accounted for approximately 75% of variability observed at sample level. The within-species variability accounted only for ~25%, and these relative contributions did not change significantly across the experiment (Table 2).

Table 2. Coefficient of variation (CV) for LMA and LNC observed within samples (n=5 for treatments and n=15 for control) and analysis of the contribution of the between species variability and the within species variability to the observed variation of LMA according to Leps *et al.* (2006). When applicable values are average ± standard deviation.

Treatment	**CV LNC (%)**	**CV LMA (%)**	**Decomposition of the observed variability in LMA***		
			Between species contribution (%)	**Within-species trait variation contribution (%)**	**Average number of species per sample considered**
Control	17-32	26-42	75.30 ± 3.46	24.70 ± 3.46	13
Drought	20-30	35-45	80.17 ± 8.90	19.83 ± 8.90	11
Heated	37-49	37-79	72.35 ± 13.25	27.65 ± 13.25	12
Watered	29-34	31-48	81.43 ± 6.24	18.57 ± 6.24	13

*The contribution of the between species and within species variability was estimated using only between 10- 14 dominant species within community and using the same number of samples per treatment and control (n=5). No significant differences were observed between treatments and control for the LMA partitioning.

Community level traits (LT_{agg})

The significant correlation observed between LMA and LNC at species level, was not observed when the data were considered at community level (r=-0.260, p=0.165, n=30), perhaps due to the different relationships observed for these traits within treatment (Figure 1) (non-significant for the Drought and the Control). LMA_{agg} (g m^{-2}), LNC_{agg} (mg N g^{-1}) and C:N $ratio_{agg}$ (Figure 3) were characterized by an important within-treatment variation (CV: LMA_{agg} 12-25%, LNC_{agg} 4-16% and C:N $ratio_{agg}$ 6-18%), being lowest in the Watered Treatment and highest in the Heated Treatment (except for LMA). Compared to the Control, LMA_{agg} showed no significant difference across treatments except for the Drought Treatment (20% higher, p<0.05, Simple contrasts in nlme); LNC_{agg} was approximately 10% lower in all the treatments and significantly different from the Control in the Drought and the Heated Treatments ($p<0.05$, Simple contrasts in nlme).

The C:N ratio$_{agg}$ followed a similar pattern to the LNC$_{agg}$ but the three treatments were significantly higher (10-20%) than the Control ($p<0.001$ for Drought and the Heated and $p<0.05$ for Watered, Simple contrasts in nlme). It is likely that the observed pattern in C:N ratio was mostly driven by variation on LNC$_{agg}$ content across the experiment, as Leaf carbon content (LCC) was constrained between 42% and 44% and did not change significantly across the experiment. No significant relationship was found between the LT$_{agg}$ and soil depth.

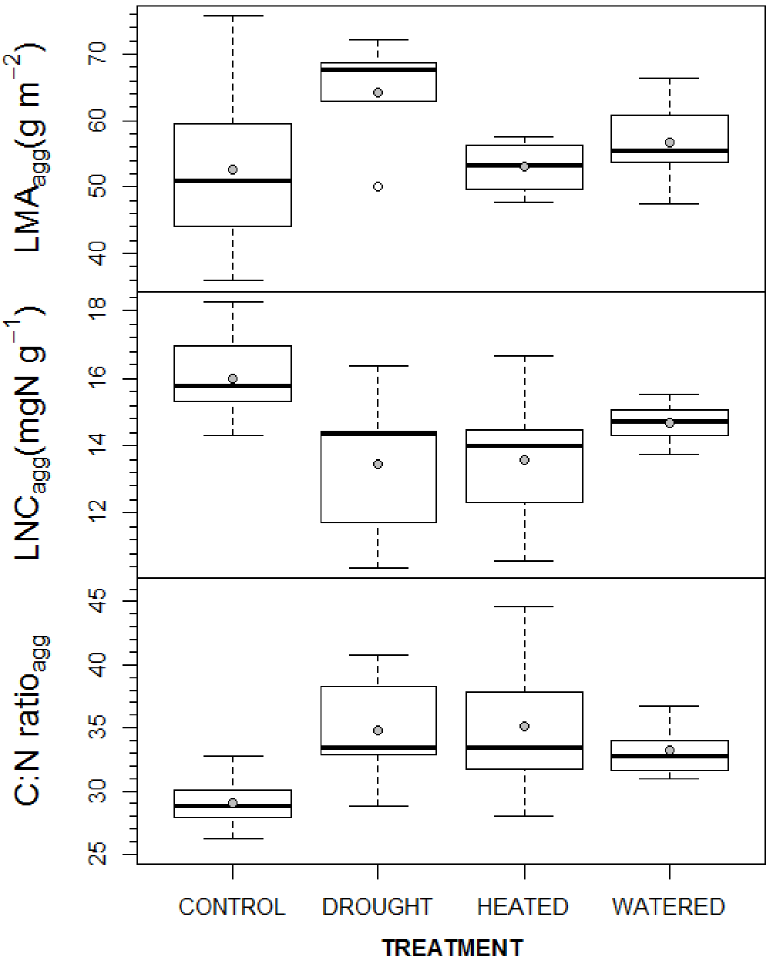

Figure 3. Community weighted means for leaf mass per area (LMA$_{agg}$), leaf nitrogen content (LNC$_{agg}$), and foliar C:N ratio (C:N ratio$_{agg}$). Each box displays the median, upper and lower quartiles of the respective distribution. Box whiskers represent the maximum and minimum range of the data. Means of the distribution are represented by grey dots. Aggregated means were estimated for each unit with (Equation 1). n=15 for control plots and n=5 for treatments. LMA$_{agg}$ was significantly different for the Drought treatment ($p<0.05$). LNC$_{agg}$ was significantly different for the Drought and the Heated treatments ($p<0.05$). C:N ratio$_{agg}$ was significantly different for all treatments ($p<0.001$ for Drought and Heated,$p<0.05$ for Watered treatment). All comparisons (simple contrasts in nlme) carried out against control treatment.

Canopy level traits

Consistent with previous observations for the site (Grime *et al.*, 2008), there was non-significant difference for the above ground biomass across the treatments (Table 3), except for the drought treatment. The drought treatment had approximately 30% less aboveground total biomass (Biomass$_{\mathrm{TOTAL}}$) than the Control ($p<0.05$, simple contrasts in nlme). The same tendency was observed for leaf biomass (Biomass$_{LEAF}$). TFN and the LAI (Table 3) followed the same pattern as biomass, and in both cases the Drought Treatment was the only one significantly different from the control (approximately 35% less TFN and 40% less LAI, $p<0.005$ and $p<0.001$ respectively for simple contrasts in nlme). Although the Watered treatment and the Heated Treatment showed marginal lower values for TFN (-8%) and LAI (-10%), the differences were not significant. The amount of litterfall on the samples represented on average 59.8 % (±11.6 standard deviation) of the entire biomass collected on the samples (Biomass$_{TOTAL}$ + Litterfall). The lowest amount of litterfall was found at the Watered treatment, which was 20% lower than the Control and 26% lower than the Drought treatment, but not significant differences were found. No significant relationship was found between the canopy traits and the soil depth except for LAI ($r=0.488$, $p<0.05$).

Table 3. Total foliar nitrogen (TFN), leaf area index (LAI), above ground biomass (total and leaves only) and litterfall for the experiment. Each data point is the mean and the standard deviation (in parenthesis). n=5 for treatments and n=15 for control.

Treatment	**TFN ($g\ N\ m^{-2}$)**	**LAI ($m^2\ m^{-2}$)**	**Biomass$_{TOTAL}$ ($g\ m^{-2}$)**	**Biomass$_{LEAF}$ ($g\ m^{-2}$)**	**Litterfall ($g\ m^{-2}$)**
Control	3.6 (1.0)	4.9 (1.4)	282.5 (85.3)	236.5 (54.9)	383.4 (196.6)
Drought	2.2 (0.7)*	2.9 (0.4)*	192.1 (54.9)*	166.4 (28.6)*	404.2 (96.3)
Heated	3.3 (0.7)	4.8 (0.6)	275.0 (52.3)	251.9 (40.4)	346.1 (139.9)
Watered	3.2 (0.7)	4.4 (1.2)	248.8 (50.1)	224.6 (46.4)	297.4 (123.8)

*The drought treatment was the only significantly different from the control ($p<0.05$ for biomass and TFN and $p<0.001$ for LAI, simple contrasts in nlme).

TFN, LAI and leaf total biomass showed a strong positive coupling across the experiment. Although, the relationship between leaf/total biomass and TFN (Biomass$_{LEAF}$: $R^2= 0.83$, $p<0.001$, $\beta=0.016$ and Biomass$_{TOTAL}$: $R^2= 0.82$, $p<0.001$, $\beta=0.011$) was stronger than between LAI and TFN ($R^2=0.59$, $p<0.001$, $\beta=0.71$). As expected, the relationship between LAI and Biomass$_{TOTAL}$ was weaker ($R^2=0.39$, $p<0.001$, $\beta=0.011$) than with Biomass$_{LEAF}$ ($R^2=0.57$, $p<0.001$, $\beta=0.020$). No significant change in R^2 or slope was observed for the relationship between LAI and Biomass$_{LEAF}$ or between TFN and Biomass$_{LEAF}$ when the model was fitted with or without intercept. TFN and LAI were fitted against Biomass$_{TOTAL}$ considering only a regression with an intercept as TFN and LAI could be zero even when there is still Biomass present.

The relationship between TFN and LAI (Figure 4) was significant for all treatments and for the entire data set (R^2=0.59), and no significant difference was observed between the slopes among treatments. The relationship between both variables is described by the common slope of 0.71 g N m^{-2} leaf area. No significant relationship was found between any of the canopy traits and species richness (number of species in the community).

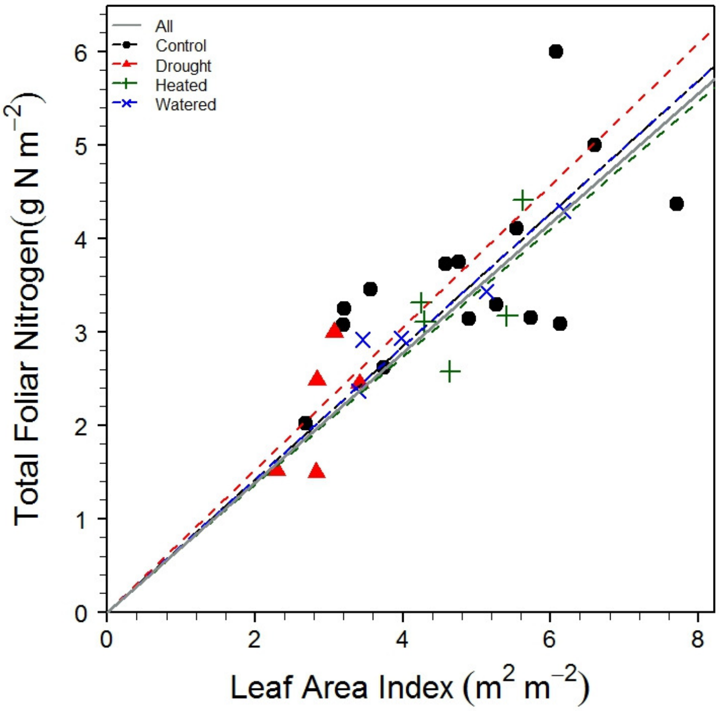

Figure 4. Relationship between LAI and TFN. All regression lines were forced through the origin and they were significant p<0.001. **Control**: β=0.71, R^2=0.43, p<0.001, n=15. **Drought Treatment:** β=0.76, R^2=0.0.43 n=5. **Heated Treatment**: β =0.68, R^2=0.36, p=<0.001, n=5, **Watered Treatment**: β =0.71, R^2=0.91 n=5. The general regression for the entire data set (**All data,** grey line) was fitted using a mixed effect model to account for the variability introduced by the pseudoreplication on the control plots. This reduced the relative mean square error (RMSE) from 0.64 to 0.52 and the improved the R^2 from 0.59 in the linear regression (β=0.71, n=30, p<0.001) to 0.67 (β=0.69, n=30, p<0.001) in the mixed effect model. Slopes were not significantly different.

Relationship between canopy level traits and leaf traits (LT_{agg}) at community level

Although TFN, biomass and LAI were strongly coupled, they were less strongly coupled to the leaf traits (LNC, LMA) that determine them at community level (Table 4). TFN as expected had a positive significant relationship with LNC_{agg} but there was no significant relationship with LMA_{agg} or with leaf nitrogen content when it was expressed on an area basis (LNC_{agg} g N m^{-2}) (despite the fact that TFN could be estimated as LNC in g N m^{-2} x LAI). LAI is the only parameter that was related to the three LT_{agg} parameters, showing a positive relationship to LNC_{agg} and negative relationship with LMA_{agg} (as expected in part from the link between TFN and LAI). Interestingly, $Biomass_{LEAF}$ and $Biomass_{TOTAL}$ did not show a significant relationship with any of the LT_{agg}.

Table 4. Results of the linear regressions between TFN and LAI and leaf traits at community level (Leaf $traits_{agg}$). Leaf traits where averaged for each sampling point using a weighted average (see Equation 1).

Canopy traits		Leaf $traits_{agg}$ LMA (g m^{-2})	LNC (mg g^{-1})	Area based LNC (g N m^{-2})
TFN (g N m^{-2})	Slope	NS	1.18	NS
	Intercept		11.1	
	R^2		0.34***	
LAI (m^2 m^{-2})	Slope	-5.18	0.524	-0.463
	Intercept	79.9	12.6	1.02
	R^2	0.313**	0.12**	0.11**

***p<0.001, **p<0.05, n=30.

Dominant species and their role on canopy traits

Across the experiment, species were considered dominant if they represented at least 25% of the total biomass, alone or in combination with the second most dominant species (20% of the samples). Across the 30 samples examined, a total of 13 species were considered dominants (Table 5) and they were characterized by relatively high values of LMA or LNC (Table S 1). Despite large variation on the LMA (33.7-120.43 g m^{-2}) and LNC (7.35-31.04 mg N g^{-1}) of the dominant species, no significant differences were observed in these parameters (Tukey Test at 95% confidence), and both parameters were inversely correlated (r=-0.4, p<0.05).

The relationship between the TFN of dominant species (TFN_{DOM}) and the LAI of dominant species (LAI_{DOM}), was highly significant ($R^2=0.62$, $p<0.001$) although the slope (0.83 g N m^{-2} LAI) was stepper than when all species were considered (0.71 g N m^{-2} leaf area) (Figure 4), no significant difference among the slopes was observed. Across the experiment (Table 5) the dominant species represented a similar ratio (not significantly different) of dominant to total TFN (30%) and of dominant to total LAI (25%).

Table 5. Total foliar nitrogen (TFN_{DOM}) and Leaf area index (LAI_{DOM}) for dominant species only. Ratio of dominant to total TFN (TFN_{DOM}:TFN) or LAI (LAI_{DOM}:LAI) was estimated as the ratio of the dominant species TFN/LAI to the value at the community scale (expressed as percentage). All values are means and standard deviation (in parentheses). Percentage of dominance is the ratio of the above-ground biomass of the dominant species to the total value of biomass of the community (see bottom of the table for details about species). n=5 for each treatment and n=15 for control.

TFN_{DOM} (g N m^{-2})	**TFN_{DOM}:TFN (%)**	**LAI_{DOM} ($m^{-2}m^{-2}$)**	**LAI_{DOM}:LAI (%)**	**Dominance (%)**	**Dominant species**
					Control
1.1 (0.6)	30.0 (7.9)	1.3 (0.6)	25.9 (7.0)	31.7 (10.1)	*Helictotrichon pratense*[a,c], *Helianthemum nummularium*[a,c], *Lotus corniculatus*[b], *Succisa pratense*[b,c], *Potentilla erecta*[a], *Plantago lanceolata, Sanguisorba minor*[c] ,*Carex caryophyllea*
					Drought
0.7 (0.5)	29.1 (12.6)	0.7 (0.2)	23.8 (6.2)	30.1 (13.0)	*Helianthemum nummularium*[b,c], *Sanguisorba minor*[b,c], *Briza media*
					Heated
1.0 (0.4)	29.2 (10.4)	1.3 (0.3)	27.7 (3.9)	33.3 (14.5)	*Festuca ovina*[a,c] , *Plantago lanceolata*[c], *Potentilla erecta*[c], *Briza media, Agrostis capillaris, Succisa pratense**
					Watered
0.8 (0.4)	23.1 (10.0)	1.0 (0.5)	24.0 (13.8)	26.7 (7.8)	*Festuca ovina, Agrostis capillaris*[c], *Potentilla erecta*[c], *Carex pulicaris, Plantago lanceolata*[c], *Lotus corniculatus*[c], *Succisa pratense**

[a] Species was dominant on 3 or more samples within treatment. [b] Species was dominant in 2 samples within treatment. [c] Species account for a relative dominance of approximately 30% in combination with other species. *in block E

Discussion

Patterns of variation in LMA and LNC

At species level, the data do not support the hypothesis (H1.1) that LMA would be higher under drier conditions and LNC would be relatively stable. While there is some suggestion that species could be following predicted patterns of variation (i.e. higher LMA and lower LNC in drought plots and lower LMA and higher LNC on water plots, Figure 1) the results are not conclusive because the LMA-LNC relationship was not significant at treatment level (at least for the drought plot), so our alternative hypothesis (H1.2) is better supported.

Although LNC showed an important level of variability across the experiment (CV 32%), its value at species level was generally more constrained than LMA, so it is likely that large variations in LMA, up to 5 times in some species (i.e. *F. ovina* and see Table S 1), are affecting the LMA-LNC relationship. Especially considering that LMA is a highly plastic trait in response to light and nutrient limitations (Poorter *et al.*, 2009, Wright *et al.*, 2004), and that it is probably changing as part of the high genetic variability that have been already described for the site (Bilton *et al.*, 2010, Whitlock *et al.*, 2011). This high genetic variability further explains the large interspecific contribution to the within-sample LMA variation found on our analysis where between-species variation on LMA accounts for 80% of the observed within sample variability (Table 2), what partially supports our hypothesis (H2.1).

Despite the high level of within-sample variation of LMA (Table 2) and the high within-treatment LMA_{agg} variability (CV 12-25%), results at community level (LT_{agg}), showed slightly clearer patterns. In the Drought treatment, the higher LMA_{agg}, likely due to the predominance of stress tolerant species (Grime *et al.*, 2008) with thicker leaves and high N content per leaf area (Hodgson *et al.*, 1999, Poorter *et al.*, 2009), partially supports our hypothesis of higher LMA under water limited conditions (H1.1). However, contrary to expectations, in the Heated treatment LMA_{agg} was similar to the Watered treatment (relatively lower) but for LNC_{agg} it was similar to the Drought treatment (Figure 3). These contradictory results

found on the Heated treatment could be partly explained by the dominance of different species, with large differences in LMA, across blocks (*F. ovina, Plantago lanceolata, Briza media*); or by the presence of relatively older leaves (probably higher LMA and lower LNC), as the Heated treatment advances the growing season (Grime *et al.*, 2000).
At community level our results suggest that although there is a clear effect of the treatments on LNC_{agg} (Figure 3), the mechanisms by which LNC_{agg} is moderated in the experiment are not entirely dependent on water availability despite the recognised role of water on N availability (McCulley *et al.*, 2009, Fridley *et al.*, 2011), and explains perhaps why we did not find any relationship between soil depth and traits at community or canopy level. Therefore the alternative hypothesis (H1.2) of variations in LNC despite N limitations (Fridley *et al.*, 2011) is supported.

Nonetheless, our findings do suggest an important role of species composition on the results found for LNC_{agg} across the experiment. For instance, we found that in all the treatments LNC_{agg} shifted in the same direction from the Control, what is likely caused by the differences in relative abundances of the species in the communities, particularly the reduction of *Lotus corniculatus* (the only N-fixing species) in all treatments. Additionally, the Drought treatment had a higher abundance of *H.nummularium* and *Sanguisorba minor* (species with relatively higher contents of LNC) and the Watered treatment presented a more even combination of functional groups (Table 1), with important presence of species with relatively lower concentrations of nitrogen (i.e. *Agrostis capillaris*, *F. ovina*); that further highlight the role of species composition on the observed patterns.

LAI-TFN regulation

Several lines of evidence support our hypothesis (H3.1) that the variation of LAI and TFN in the ecosystem is driven by the amount of biomass and the characteristics of the dominant species in the community. First, the observed reduction on TFN and LAI in the drought plot (Table 3), coincided with the reduction on biomass at this treatment (as previously reported by Grime *et al.* (2008) and Fridley *et al.* (2011)), result additionally supported by the strong relationship between $Biomass_{LEAF}$ and TFN (R^2=0.83). Second, we found that in 80% of the communities examined one species could represent on average 30% of the total biomass, and that the TFN and the LAI of this dominant species represented a consistent ratio to the total TFN or LAI across the experiment (Table 5). This second finding is perhaps explained by the fact that most of the dominant species (i.e. *H. nummularium, F. ovina, H. pratense, S.minor, L. corniculatus*) regardless of their functional type have similar values of their individual leaf traits (Table S 1), which are not significantly different across treatments. Third, we found that using only the data from the dominant species we could estimate the slope of the TFN-LAI relationship (0.83 g N m^{-2} leaf area) and that this slope was not significantly different from the one considering all the species (Figure 4). This, has important practical implications for the scaling up of the results found on this ecosystem with relatively good confidence (R^2=0.59, RMSE=0.86).

Although we expected that the TFN-LAI relationship will be affected by the treatments, our results suggest that the TFN-LAI relationship (Figure 4) is equally coupled across treatments (no significant difference between slopes was observed), and that is likely that the species optimize the relative amounts of foliar N and leaf area within the same constraints (Williams and Rastetter, 1999), falsifying our hypothesis (H3.1) . However, our data also suggests that there is an important shift along the same slope, and that this shift is largely controlled by changes in LAI, evident in the drought plot where a reduction on LAI, an adaptation to the drier conditions (Chapin III *et al.*, 2002), limits the maximum attainable TFN.

Our results also suggest that the existence of a trade-off between LMA and LAI in the treatments, as a strategy to maximize LAI (for light capture), up to the point that it can be sustained by the available resources and it does not mean the loss of other properties more important for persistence (i.e. leaf toughness) (Hikosaka, 2005, Nyfeler *et al.*, 2009). This trade-off is likely could possible explain some of our results especially considering that TFN (estimated here as LNC x $\text{Biomass}_{\text{LEAF}}$) could be expressed in terms of LMA and LAI, where LMA x LAI could replace the $\text{Biomass}_{\text{LEAF}}$ term as suggested by van Wijk *et al.* (2005). Further implying that treatments dominated by species with high LMA and relatively high LNC (more N on area basis), i.e. *H. nummularium* and *S. minor* are likely to show a larger increment on TFN by a unit of change on LAI.

Community stability and implications for N economics

Although this study was not set up particularly to explore resource limitations in the ecosystem, at the different levels of aggregation explored in this paper (leaf level, community level and canopy level), our results suggest that the subtle responses observed are likely due to the combination of relatively stable communities and strong regulation at species level and within the assemblage to maximize productivity under limited resources (N, P, water or even light).

At leaf level, the way the slope of the LMA-LNC relationship significantly differed when species were grouped in their functional type (grasses, sedges and forbs) (Figure 2) suggests differences in N allocation patterns and in leaf orientation (vertical in grasses and semi-horizontal in forbs). This strategy is perhaps more evident in the forbs, where low LMA and high LNC leaves maximize photosynthesis (a shade tolerance response), although the existence of a "tail" in the forbs LMA-LNC relationship (Figure 4) is influenced by legumes, they represent only a 16% of the total of individuals included in this analysis and their influence on the slope of this relationship is negligible. As the pattern of this relationship for forbs is generalised across treatments (compare Figure 1 and Figure 2), this is likely an strategic adjustment and not only the result of differences in leaf age (Kikuzawa and Lechowicz, 2011) that could be eventually caused by an earlier flushing in the heated treatment.

At community level, although the similar amount of biomass and its similar quality (average C:N ratio 30.6 ± 2.1 across the experiment, see Figure 3), might suggest the same speed of decomposition; the difference in the amount of litterfall present in the treatments (from 48% in the watered plot to 60% drought plot litterfall/Biomass$_{\mathrm{TOTAL}}$+ Litterfall) (Table 3) suggests that the treatments might have affected the fragmentation of the materials and the activity of the decomposers (Grime *et al.*, 1996). The effect of the treatments might be through a faster turnover or a tighter recycling of N (in the watered plot), through the increment on decomposition rates through higher temperatures (in heated plot) or by slowing decomposition rates through the presence of more recalcitrant material (higher LMA$_{\mathrm{agg}}$ in the drought treatment). Although our results for litterfall could include more than one year of litterfall and they are not significantly different from control, they might have important implications in terms of nutrient recycling, particularly considering that the C:N ratio from leaves could be up to 50% higher as N is removed slowly previous to leaf shedding (McGroddy *et al.*, 2004), so this is an aspect that should be further explored.

At canopy level, although our values of TFN (1.5 to 6 g N m^{-2}), are higher than values reported for other natural ecosystems like Tundra (between 1.4 and 2 g N m^{-2} reported by Street *et al.*(2012), Campioli *et al.*(2009), and van Wijk et al. (2005)), or prairies (between 1.0 and 2 g N m^{-2} reported by McCulley *et al*. (2009)); the slope of the TFN-LAI in our results 0.71 g N m^{-2} leaf area (Figure 4) is low in comparison to the slope reported on these studies (i.e. slope near 1.90 for LAI < 3). This lower slope but higher TFN suggests that TFN in this ecosystem is likely the result of a tighter N cycling, particularly on the watered plot (McCulley *et al.*, 2009), and that is likely achieved in the experiment (particularly the watered plot) through the high species richness and that allows for a more effective exploration/use of resources.

Conclusions

This study has shown that the high plasticity of LMA in response to changes in the environment, and the strategies of species for resource optimization under limiting conditions (Grime, 1977) are essential to maximize productivity (evaluated as TFN) within the available resources. The differences observed in species abundance suggest that if a group with high investment in support structures (shrubs and forbs) is dominant or if species with a low $\text{Biomass}_{\text{LEAF}}$/total ratio (i.e. grasses) are quite dense, the trade-off in LMA-LAI could potentially lead to a more curved relationship between TFN and LAI, suggesting that N concentration per leaf area decreases at high LAI, and impacting leaf turnover and nutrient cycling. This curvature could possibly be evaluated with a larger number of repetitions including samples at both ends off the LAI (LAI< 2 or LAI >6).

The relatively stable composition of the communities (same % of within community variability for LMA across the experiment) and small changes in species richness, support the idea that the treatments' effect on the aboveground properties is mostly through their effect on species composition (their identity but primarily their relative abundance) (Grime, 1998). Although our within-community results need to be interpreted with caution as it was only evaluated for LMA in 10-12 dominant species, it is possible that this pattern could be a generalized even if more species were included; as the proportion of between/within species variation remained quite constant across the 30 communities examined.

Despite the relevance that the dominant species have on our results and on the prediction of the canopy level properties, it is likely the nutrient cycling on this ecosystem depends also on the identity of other non-dominant species within the community, their ability to explore different resources and their effects on the activity of the microbial community (Scherer-Lorenzen *et al.*, 2003), so diversity might still play an important role in the efficient use of resources. Therefore, taking functional diversity into account may be a necessary part of predicting the relationship between plant traits and ecosystem function, particularly in disturbed or less productive systems (Pakeman *et al.*, 2011).

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Table S 1. LMA and LNC for the 23 most abundant (common) species. Values are average and standard deviation along the experiment. Coefficients of variation (CV) are the variation of the trait along the experiment expressed as %. Frequency denotes species occurrence in the 30 samples. % Dominance was estimated as the total biomass of the species/total biomass of the sample. Max dominance and Average dominance refers to the maximum dominance (estimated from biomass) observed for the species in a single sample and the average dominance across the experiment, respectively.*Within species variability at the experiment level.

Species	**Functional Type**	**LMA (g m^{-2})**	**LMA CV (%)***	**LNC (mgN g^{-1})**	**LNC CV (%)***	**Frequency**	**Maximum Dominance (%)**	**Average dominance (%)**
Agrostis capillaris	Grass	36.08 ± 9.60	26.60	13.39 ± 1.57	11.76	30	25.5	6.1
Anthoxanthum odoratum	Grass	35.98 ± 11.89	33.05	14.95 ± 1.37	9.17	26	8.8	2.4
Briza media	Grass	45.34 ± 14.14	31.18	12.83 ± 1.37	10.72	21	25.5	7.4
Campanula rotundifolia	Forb	29.36 ± 10.65	36.27	16.07 ± NA	NA	21	< 1	0.4
Carex flacca	Sedge	55.95 ± 10.70	19.12	13.27 ± 3.53	26.61	30	20.6	5.4
Carex panicea	Sedge	57.08 ± 12.68	22.22	13.21 ± 1.36	10.31	29	15.5	7.2
Carex pulicaris	Sedge	80.46 ± 23.51	29.22	11.87 ± 1.02	8.60	13	18.0	5.7
Carex caryophyllea	Sedge	41.62 ± 8.43	20.24	13.65 ± 1.15	8.44	29	19.5	5.3
Danthonia decumbens	Grass	41.70 ± 13.52	32.43	13.64 ± 1.07	7.88	23	7.7	1.3
Festuca ovina	Grass	82.62 ± 34.27	41.48	10.36 ± 1.61	15.53	30	33.0	9.0
Helianthemum nummularium	Suffrutescent shrub	62.95 ± 21.50	34.16	19.42 ± 0.86	4.40	12	57.4	16.5
Helictotrichon pratense	Grass	65.43 ± 19.73	30.16	12.63 ± 0.85	6.71	27	36.8	11.0
Hypericum pulcrum	Forb	47.98 ± 12.11	25.24	13.73 ± 2.32	16.89	17	4.9	0.8
Koeleria macrantha	Grass	51.79 ± 13.87	26.78	14.17 ± 1.73	12.19	28	12.6	5.5
Linum catharticum	Forb	38.20 ± 17.35	45.40	17.71 ± 4.79	27.04	16	2.5	0.4
Lotus corniculatus	Forb	41.32 ± 7.63	18.47	26.47 ± 4.18	15.78	27	19.7	7.2
Plantago lanceolata	Forb	60.63 ± 21.09	34.79	13.97 ± 2.94	21.05	27	27.4	7.9
Potentilla erecta	Forb	41.49 ± 8.88	21.39	16.49 ± 2.64	16.01	28	30.4	8.8
Polygala vulgaris	Forb	37.26 ± 10.27	27.57	23.07 ± 3.22	13.96	6	3.6	0.8
Sanguisorba minor	Forb	39.33 ± 6.53	16.61	19.43 ± 1.24	6.38	22	18.3	5.7
Scabiosa columbaria	Forb	46.73 ± 13.34	28.54	16.21 ± 3.10	19.13	22	5.3	2.0
Succisa pratensis	Forb	70.76 ± 16.25	22.96	10.63 ± 2.43	22.89	5.00	33.7	23.2
Viola riviniana	Forb	36.53 ± 11.75	32.17	13.58 ± 1.81	13.35	23	3.7	0.8

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Chapter 3a. Soil moisture, leaf area index and inter-annual climatic variability control gross primary productivity in limestone grasslands in a field-scale climate manipulation experiment

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Abstract

Climate manipulations experiments established in the last decades have proven to be an important tool to identify and quantify mechanisms controlling ecosystem functioning under our changing climate. One of these long-term experiments established in Buxton, UK, has exposed species-rich grasslands to increased winter temperature (+ 3 ºC), or summer rainfall addition/exclusion, for over 15 years. However, at this site no major changes have been observed in net primary productivity (biomass). To improve our understanding of the mechanisms that confer this relative stability, we explored how the interactions among species composition, soil conditions and microclimate affect the carbon cycle of this ecosystem. Over the summer of 2010 and 2011 we measured net CO_2 light response curves and estimated gross primary productivity (GPP) and ecosystem respiration (R_{eco}). At consistent light and temperature, GPP and R_{eco} were variable across treatments; GPP was 30% lower on the drought treatment and 30% higher on the watered treatment, and similar responses were observed for R_{eco}. The winter warming treatment was not significantly different from the control. Both GPP and R_{eco} were strongly controlled by soil moisture in interaction with biomass (leaf area index), but the observed responses were likely affected by the conditions experienced by the ecosystem prior to the measurement period, particularly in the control plots where R_{eco} in 2011 was 60% higher than in 2010. Up to a 34% of the variance in GPP and R_{eco} was explained by the species present and their relative abundances. Although this research focused mainly on the effect of the treatments on the CO_2 fluxes during the peak growing season, it revealed the complexity of the drivers of GPP_{600} and R_{eco}. Despite the significant differences observed in GPP, we suggest that the stability in the net primary productivity might be explained by a higher allocation to belowground structures and the reduction of the leaf area index to withstand water stress in the drought plot, and a more rapid exchange of leaves and faster turnover of litter in the watered treatment.

Introduction

The last decades have seen the development of a number of climate manipulation experiments to test the effect of predicted changes in temperature, precipitation and CO_2 concentration on ecosystem carbon cycling (IPCC, 2007, Hulme *et al.*, 2002). So far, these type of experiments (i.e. Milbau *et al.*(2005), Vicca *et al.*(2007), Beier (2004), Fisher *et al.*(2007),van Wijk *et al.* (2004)) have shown that, although the responses observed are not generalized or unidirectional (Rustad *et al.*, 2001, Wu *et al.*, 2011), they are a valuable tool to identify and quantify mechanisms controlling the observed responses (Klein *et al.*, 2005).

One of the oldest experiments of this kind in Europe (Beier, 2004), was established in Buxton, UK, in 1993, where the selected limestone grasslands have been subjected to soil warming during the winter or to droughting/irrigation during the summer (Burke and Grime, 1996). However, only minor changes have been observed in species composition or biomass (linked to net primary productivity [NPP]), with the exception of the droughting manipulation that showed a 35% drop in NPP and changes in species composition (Grime *et al.*, 2008). The lack of clear effect of the other treatments is surprising (see Milbau *et al.* (2005), Grime *et al.*(2000) for similar experiments), given the extended growing season in the warming treatment produced by early snow melting, and the well-drained soils present at the site, where productivity is expected to increase with more available water (in the irrigation treatment).

Despite the long historical use of biomass harvest to assess ecosystem productivity (Garnier *et al.*, 2004, Scurlock *et al.*, 2002), this approach might not be adequate to fully explain what is happening with this ecosystem carbon cycle. Because the harvest method only takes into account the new plant biomass produced, it does not provide enough information about the allocation processes to roots and other plant structures (Chapin III *et al.*, 2002). Furthermore, because this method does not explore how the different fluxes involved in ecosystem productivity (inputs through Gross primary productivity [GPP] and outputs through ecosystem respiration [R_{eco}]) are affected by the treatments, which is critical for understanding both the direction and magnitude of the treatments on the carbon cycle.

Therefore, in this paper we explore how instantaneous measurements of GPP and R_{eco} are affected by the treatments, during the summer peak productivity of the experiment. We anticipate that the fluxes will show a complex behaviour as they might be affected in different temporal/spatial scales (Olivas *et al.*, 2011, Williams *et al.*, 2006), not only by the treatment but also by the interaction with other non-controlled factors (i.e. soil fertility, rainfall). Furthermore, CO_2 exchange could be influenced by small scale variations in soil moisture with microtopography (Klein *et al.*, 2005, Zhou *et al.*, 2006, Niu *et al.*, 2008), by differences in species composition (Hirota *et al.*, 2010), and by above-ground biomass properties (leaf area index [LAI] and total foliar nitrogen [TFN]) (Street *et al.*, 2007, Shaver *et al.*, 2007)). Additionally, GPP and R_{eco} though strongly linked through the C and N/P cycles (Chapin III *et al.*, 2009), could decouple if the treatments affect mineralization processes unevenly.

The specific questions we address in this paper are:

1. What is the effect of the treatments on the instantaneous values of GPP and R_{eco}? Is there a clear effect of the edaphic properties (microsite conditions) on the observed response?
   H1.1). We hypothesise that across treatments GPP and R_{eco} are coupled and they will be affected in the same direction, and that compared to the control they will be higher where the resources are less limiting (i.e. watered treatment). Additionally we expect to observe a clear role of the edaphic properties on the observed response where shallow/water limited soils will be less productive (i.e. drought treatment).
   H1.2). Our alternative hypothesis is that the treatments decouple GPP from R_{eco}, particularly in shallow soils where plants might have developed different allocation strategies (i.e. deeper roots) so the creation of temporal microclimatic conditions (i.e. drier soils) might not have a significant impact on GPP but might affect R_{eco} as the soil dries.

2. Can the changes in GPP be linked to above-ground biomass properties, particularly related to light absorption capacity and leaf nutrient content?
   H2.1). We expect a strong link between GPP and above ground biomass traits (i.e. LAI, vegetation height, total foliar nitrogen), however, the observed response is

likely to be the result of interaction between above ground properties and their effect on microenvironment conditions (temperature, soil moisture).

H2.2). Our alternative hypothesis is that there is a strong relationship between GPP and LAI, and that this relationship is not affected by soil conditions.

3. Is there evidence of an effect of species composition on GPP and R_{eco}?

   H3.1). We hypothesise that GPP and R_{eco} will be strongly linked to species composition (their identity, their abundance and their traits).

   H3.2). Alternatively, we hypothesise that the fluxes could be more strongly linked to species richness (regardless of their traits), as this is essential to buffer climate change.

Although it is still unclear if grasslands will act as sinks or sources of CO_2 under predicted climate change (Niu *et al.*, 2008, Scurlock and Hall, 1998), it is likely that their response to climatic drivers will be spatio-temporally heterogeneous at small scales (Risch and Frank, 2007). The experiment in Buxton, where microtopographic changes are clearly identified (Fridley *et al.*, 2011, Moser *et al.*, 2011), provides an opportunity to explore the mechanisms governing gas exchange and their microscale variations. This analysis will improve our understanding on how the interactions among species, microclimate and soil conditions affect CO_2 dynamics, and how they may feedback to the global carbon cycle; something relevant considering that grasslands cover almost 30% of the world land surface (Janssens *et al.*, 2005, Jones and Donnelly, 2004).

Methodology

Study site and treatments

The field site is located at the Buxton Climate Change Impacts Laboratory (BCCIL) at the main facility of the Health and Safety Laboratory in Harpur Hill, in Buxton, Derbyshire, UK. Full details on location, soil properties and historic climatic conditions are described in Chapter 2. The main experiment is composed of 5 (10 x 10 m) blocks of treatments on a northwest facing slope of approximately 35°. Each block is subdivided into 9 (3 x 3 m) plots where the climatic manipulations have been carried out since 1993, as described in detail in Grime *et al.* (2008). In summary, at the heated treatment soil temperature has been increased from November to April, by 3˚C above the ambient temperature, by the use of two 1000 W, 240-V AC heating cables secured to the soil surface in parallel runs spaced 50 mm apart. At the drought treatment, rainfall has been excluded from the plot throughout July and August by using sliding semi-transparent shelters that operate automatically when the rain starts/stops. At the watered treatment, from June to August, additional water has been added to the plots by drip irrigation to provide 20% more inputs than the long term Buxton average. Besides the three previously described treatments and the control plot, each block has two plots where the treatments have been combined (incremented winter temperature + intercepted rainfall, or incremented winter temperature + supplemented rainfall), and three unused plots. In addition to the climatic treatments, in October of each year the vegetation is cut to a 40-50 mm height to simulate natural sheep grazing and cut material is removed from the plot

Between 2006 and 2008 a total of 8 (10 x 10 cm) microsites were established in each plot to carry out more detailed research on whether the species were responding at a scale undetected by the 3 x 3 m scale. The microsite locations were stratified based on soil depth, 2 microsites were assigned to each depth strata of 0-7, 8-12, 13-20 and 21+cm (Fridley *et al.*, 2011). Each microsite was assigned with a unique number.

Sampling strategy

The CO_2 flux measurements were carried out during the July 2010 and July-August 2011, and we focus our work only on the main treatments (watered, heated and drought) and the control plots (4 plots per block). The selection of the sampling positions was a compromise between the need to position the sampling points randomly and our interest to cover the soil depth strata identified by Fridley *et al.* (2011) (named hereafter as microsites). In 2010 four (10 x 10 cm) microsites were chosen in the drought and watered treatments along four blocks (blocks B to E). To select the sampling position from each pair of soil depths (0-7, 8-12, 13-20 and 21+cm) we selected one microsite randomly (by drawing a piece of paper with the respective microsite number from a bag), a total of four microsites per plot were studied. In 2011 the measurements were carried only in two microsites per plot (the extremes of the soil depth categories i.e. 0-7 cm and 21+cm), but the three main treatments (drought, watered and heated) and the five blocks (A to E) were covered. The selection of the microsites in 2011 followed the same random procedure described for 2010. Measurements in the control plots were included in the blocks as described for the treatments in both years.

Ecosystem CO_2 exchange

A LI-COR 6400 (LI-COR Lincoln, Nebraska), operating in closed mode and coupled to a 20 x 20 cm Perspex chamber was used to estimate CO_2 exchange in the microsites. The chamber was placed on a base above the ground, the height of the base was adjusted using steel legs of different length carefully inserted in the ground. A plastic sheet was used to seal the base to the ground (the plastic sheeting was held firmly against the soil surface by a metal chain around the perimeter of the frame). The chamber was equipped with a fan to ensure appropriate circulation of the air. Further details of this measurement approach are described in Williams *et al.* (2006) and Street *et al*. (2007).

Each measurement consisted of 5 second integrations of CO_2 flux that spanned a period of 30 to 45 seconds. We calculated the net CO_2 flux (Fc) in µmols m^{-2} s^{-1} from the temporal change in CO_2 concentration according to Equation 1:

$$Fc = \frac{\rho \times V \times dC/dt}{A} \qquad \text{Equation 1}$$

Where ρ is air density (mol m^{-3}), V is the chamber CO_2 volume (m^3), dC/dt (µmols mol^{-1} s^{-1}) is the slope of the change in CO_2 concentration in time, and A is the surface area of the chamber (m^2).

Since no simultaneous measurements between plots could be performed, CO_2 exchange at each plot was measured with artificial variation in light intensity, to generate a light-response curve. The basic sequence of work included a measurement under ambient light, followed by 3 levels of shading (generated using a white optically neutral sheet) and a final measurement under dark conditions using a black sheet. The average of the dark measurements was used as a proxy for ecosystem respiration (R_{eco}). Each light level was repeated at least 3 times. Measurements were discarded when condensation formed inside the chamber or large variations of temperature during the measuring period were observed, temperature during the measuring cycle was kept within an experimental variaton of of 0.89 °C during 2010 and around 1.11 °C in 2011. Of the 91 light curves measured in 2010-2011, 90% were considered suitable for the data analysis.

Gross primary productivity (GPP) at each light level was estimated as net ecosystem production NEP (the negative of NEE) + $R_{eco.}$ The response of GPP to incident light for each microsite was modelled as a rectangular hyporbola following Street *et al.* (2007) according to Equation 2:

$$GPP = \frac{P_{\max} \times E_0 \times I}{P_{\max} + E_0 \times I} \qquad \text{Equation 2}$$

Where $P_{\max}$ is the rate of light saturated photosyntheis (µmol CO_2 $m^{-2}s^{-1}$), E_o is the initial slope of the light response curve or the quantum efficiency (µmol CO_2 µmol^{-1} PAR) and I is the incident photosynthetic active radiation (µmol PAR m^{-2} s^{-1}). $P_{\max}$ and E_o in equation 2 were fitted with a nonlinear least-square model using the software R 2.15.2 (R Core Team, 2012). Light compensation point (µmol PAR m^{-2} s^{-1}) was calculated as $LCP = (R_{eco} * P_{max}/Eo)/(P_{max} - R_{eco})$.

Environmental variables and vegetation properties

After each light curve, soil volumetric water content ($m^3 m^{-3}$) was measured using a soil moisture sensor S-SMx-M005 (ONSET, Cape Cod, Massachusetts, USA) and soil temperature (°C) was measured with a traceable thermometer. Both measurements were done at 5 cm of soil depth in 3 to 5 positions within the 100 cm^2. Vegetation height was measured at 5 different points within the microsite after each gas exchange measurement cycle finished (only in 2011). Weather data were obtained from the weather station located in Harpur Hill, at 53°14’38’’N, 1°54’11’’W and 340 m.a.s.l.

Since harvesting of the above ground biomass was not possible at the microsites, we used estimates of leaf area index (amount the leaf area on a square meter-LAI) and the normalized vegetation index (NDVI) as proxies of above-ground biomass. LAI at each microsite was indirectly measured using a LI-2000 (LI-COR Lincoln, Nebraska, USA) and values were recalculated with the Lang Method recommended for short canopies (LI-COR, 2011). NDVI was measured using a Spectrosense 2 (Skye Instruments Limited Powys, UK).

Additional variables useful in the interpretation of the results were provided from the long term records at the BBCIL including soil depths (estimated as described in Fridley *et al.*(2011)). Total cover of vascular plant species in year 2010 and 2011 was available only for the control plots, so the vascular cover of 2012 was used as proxy for plant cover for the 2011 estimations in the drought, heated and watered plots.

We estimated a community leaf nitrogen content mean (LNC_{agg}) for each microsite in the 2011 data set and for controls as well in 2010 according to Equation 3:

$$LNC_{agg} = \sum_{i=1}^{n} p_i \times N_{content} \qquad \text{Equation 3}$$

where p*i* is the relative contribution of species *i* to the maximum plant cover of the microsite following Garnier *et al.* (2004), *pi* was estimated from the plant cover data as previously described. $N_{content}$ was estimated from a survey carried out in 2010

(Chapter 2), and when possible leaf nitrogen content (LNC) values were assigned considering species from the same treatment. When species information was not available, LNC values were estimated prior to the estimation of LNC_{agg} using nearest neighbour distance hot-deck approach in the StatMatch package (D'Orazio, 2012) with functional group as the class for data imputation.

Total foliar nitrogen (TFN g N m^{-2}) was estimated from a multiple regression (not forced through the origin) including LNC_{agg} and indirect measurements of LAI, against TFN estimations from the direct harvest carried out in 2010 (Chapter 2). The equation (see equation 4 in result section) was then used to estimate TFN for the microsites considering indirect LAI (measurements from the LAI-2000) and LNC_{agg} (from Equation 3). TFN was for all microsites in 2011 and for microsites only in the control plots in 2010.

Data analysis

GPP and R_{eco} were standardized across measurements using a set value of incident light for GPP (600 µmol m^{-2} s^{-1} of PAR) and a standard value of temperature for ecosystem respiration. Although average non-adjusted R_{eco} and air temperature within the chamber were strongly correlated, we used soil temperature to adjust the respiration to °15 C across the experiment (R_{eco15s}), as this produced a better fit than using air temperature. The original soil classes were reassigned as only 3 categories for the data analysis, the 2 extremes were kept as shallow (0-7 cm) and deep (+21 cm) and the classes in between (8-12 and 13-20 cm) were considered as medium soil depth (class only present in 2010).

To analyse the effect of the treatments on GPP_{600} and R_{eco15} we considered the data both yearly and as a combined data set. For the yearly analysis we used a mixed effect model in a split-plot design with soil category nested within the Block/Plot structure (the random term of the model) using the nlme package in the software R 2.15.2 2 (R Core Team, 2012), and Treatment and Soil category were treated as fixed effects. Then we analysed the effect of the treatments on the combined data set nesting the random structure in year. Models were compared with likelihood ratio tests for the significant contribution of a fixed year against a model including only random year effect. 2011's GPP_{600} data were analysed using a weighted regression with different variances per soil depth stratum (with VarIdent in R) to account for the heterogeneity of variance (Zuur *et al.*, 2009) found in the different soil categories (shallow and deep). A similar statistical approach was followed to compare the effect of treatment on the R_{eco15s} in the entire data set with a different variance structure per treatment in 2011. Pseudo R^2 values for the fixed (marginal) and fixed+random effects (conditional) were estimated using the approach described by (Nakagawa and Schielzeth, 2013) implemented in the MumIn package in R (Barton, 2013). Simple comparisons of means between treatments and control plots were carried out using the "contrasts" option of R within the mixed effect model.

To explore how physiologically important environmental factors and vegetation characteristics could control carbon uptake, we analysed the relationships between the driver variables and the GPP_{600} using a multiple regression and a mixed effect model (with Year/Block/Plot as random variables). We included the interaction between soil moisture and LAI as soil moisture and above ground biomass (measured in our case as NDVI or LAI) had been reported to interact in the control of the instantaneous measurements of *GPP* (Dagg and Lafleur, 2011, Street *et al.*, 2007). Mixed effect models were compared with likelilhood ratio tests for the contribution of the factors in different order (i.e. soil moisture before LAI or viceversa), in addition to the reduction of the Akaike information criterion (AIC). The order of the factors in multiple regression was selected using backwards and forward stepwise regression. We used non-parametric smoothers (Figure 4) to identify curvature in the relationship between variables, and included a term to account for curvature when needed.

The validity of the mixed effects analysis was checked with likelihood ratio tests comparing the models with fixed effects to the null models with only the random effects (if the model including fixed effects did not differ significantly [$p<0.05$] from the null model the results were rejected). In all cases the final model selected was fitted with REML (reduced maximum likelihood estimation). We used mixed effect models when possible because of the advantages that they have over the classical least squares approach regarding better handling of missing values, parameter estimation and prediction (Hector *et al.*, 2011).

To explore the role of species composition on the fluxes we only used the flux data from 2011 (when the more recent plant cover survey data was available for the treatments). First, we explored the role of species composition using multiple regression of non-metric multidimensional scaling (NMDS) ordination axis (Borcard *et al.*, 2011). NMDS axis were generated in the VEGAN package in R (Oksanene *et al.*, 2013). The CO_2 fluxes (GPP and R_{eco15s}) were logarithmically transformed previous to the NMDS regression to improve normality of the residuals. Then, we explored the role of species richness and diversity (Simpson Index) using simple regression.

The role of nitrogen-related leaf traits in the control of the fluxes was explored with multiple regressions; first adding the contribution of the $\mathrm{LNC}_{\mathrm{agg}}$ to the most explanatory variables of the fluxes, and then considering the contribution of TFN alone. The relationship between TFN and the fluxes was explored only for the data from the controls in 2010 and 2011 given that for these treatments the survey has been carried out on the specific year, and the fact that the calibration curve was generated predominantly from samples taken from the control plots.

Results

Environmental conditions

During the measurement period in 2010 (6-28 July) the average daily air temperature remained around 14.8 °C and for the 2011 campaign (11 July-5 August) temperature was around 13.8 °C. The total precipitation for the measurement period was 87 mm in 2010 and 86 mm for 2011. Despite the relatively similar conditions in temperature and precipitation experienced during measurements in both years, there was an important difference in the amount of sunshine hours between years; for the month of July, 81 hours in 2010 and 167 hours in 2011. Additionally both year experienced important differences in the amount of precipitation received during the spring-early summer (for the April-June months): 100 mm in 2010 and 178 mm in 2011.

Soil moisture remained fairly constant across the measurement period in both years (Table 1). In 2010, compared to the control, soil moisture was 30% higher at the watered treatment (significantly different) and the drought treatment was 10% lower but not significantly different. In 2011 the drought treatment was the only significantly different from the control (45% lower).

Soil temperature did not present a significant difference between the treatments and the control on either 2010 or 2011. The only significant difference ($p< 0.05$ simple contrasts in nlme) arose when the data were combined and the drought treatment was 30% lower than the control. Additionally, the drought treatment was the only one where a relative difference between years was observed. As expected soil moisture and temperature were significantly inversely correlated across the entire data set (Table 2).

Average soil depth was similar across years but with a larger variability in the second year (CV=53% for 2011 and 49% for 2010), which is related to the fact that we included microsites on the 4 soil depth classes in the first year but only on the extremes the second year. Soil depth was weakly correlated with soil temperature but not correlated with soil moisture (Table 2).

Table 1. Leaf area index (LAI), Normalized Difference Vegetation Index (NDVI), vegetation height and soil properties (moisture, temperature and depth) evaluated in Buxton in 2010 and 2011. Values are average and standard error (in parentheses). n=40 for 2010 and n=41 for 2011. Vegetation height was not measured (n.m.) in 2010.

Treatment	Soil properties			Vegetation properties		
	Soil moisture ($m^3 m^{-3}$)	Soil temperature (°C)	Soil depth (cm)	NDVI	LAI ($m^2 m^{-2}$)	Vegetation height (cm)
			2010			
Control	0.21	17.25	14.93	0.84	1.67	n.m.
	(0.02)	(0.34)	(2.48)	(0.00)	(0.23)	
Drought	0.19	17.82	12.51	0.77	1.15*	n.m.
	(0.01)	(0.47)	(1.19)	(0.02)	(0.11)	
Watered	0.28**	15.49	14.99	0.88	2.01	n.m.
	(0.01)	(0.66)	(1.75)	(0.00)	(0.21)	
			2011			
Control	0.23	16.63	14.64	0.81	1.68	8.47
	(0.02)	(0.38)	(3.43)	(0.01)	(0.24)	(0.60)
Drought	0.13**	18.75	11.81	0.78	1.08*	8.48
	(0.02)	(0.55)	(1.66)	(0.01)	(0.14)	(0.56)
Heated	0.23	17.66	13.47	0.81	1.39	8.18
	(0.03)	(0.52)	(1.61)	(0.00)	(0.25)	(0.61)
Watered	0.26	17.40	15.42	0.85	2.43	10.58
	(0.03)	(0.55)	(2.42)	(0.01)	(0.33)	(0.67)

**Values were significantly different from the control ($p<0.05$), or *marginally different ($p<0.1$) on the respective year when evaluated through simple contrasts in nlme.

Vegetation properties

The three measurements used to characterize aboveground biomass (LAI, NDVI and vegetation height) were correlated (Table 2); NDVI was significantly correlated with LAI across years and in the entire data set, and both variables were also weakly correlated with vegetation height (measured only in 2011). A significant correlation ($p<0.001$) was also found between soil moisture and LAI/NDVI but not with vegetation height (Table 2).

Table 2. Pearson's correlation coefficients of CO_2 fluxes, environmental properties, soil and vegetation properties for the microsites evaluated in Buxton in 2010 and 2011(n=81 except for vegetation height [n=40]).

	Environmental Properties		**CO_2 Fluxes**		**Vegetation properties**			**Soil Properties**		
	PAR	**Air temperature**	GPP_{600}	R_{eco}	**Vegetation height**	**NDVI**	**LAI**	**Soil moisture**	**Soil temperature**	**Soil depth**
PAR	1									
Air temperature	0.563 ***	1								
GPP_{600}	-0.207	0.096	1							
R_{eco}	0.034	0.400 ***	0.804 ***	1						
Vegetation height	-0.257	0.024	0.413 **	0.320 *	1					
NDVI	-0.163	-0.123	0.568 ***	0.347 **	0.374 *	1				
LAI	-0.016	-0.007	0.318 **	0.300 **	0.368 *	0.451 ***	1			
Soil moisture	-0.151	-0.201	0.525 ***	0.378 ***	0.190	0.426 ***	0.292 **	1		
Soil temperature	0.251 *	0.555 ***	-0.124	0.077	0.057	-0.344 **	-0.137	-0.462 ***	1	
Soil depth	-0.027	-0.195	0.122	-0.059	-0.075	0.324 **	0.243 *	0.204	-0.218 *	1

***$p<0.001$,**$p<0.01$, *$p<0.05$. PAR (µmol PAR m^{-2} s^{-1}), is the maximum photosynthetic active radiation in the measurement cycle, air temperature (°C) is the average of the temperature measured inside the chamber for the 9 points of the light curve in the light, GPP_{600} (µmol CO_2 m^{-2} s^{-1}) is the GPP estimated at 600 PAR, R_{eco} (µmol CO_2 m^{-2} s^{-1}) is the average of the ecosystem respiration measurements at each microsite not temperature-adjusted. NDVI and LAI (m^2 m^{-2}) are point measurements in each microsite. Vegetation height (cm), Soil moisture (m^3 m^{-3}), Soil temperature (°C) and Soil depth (cm) are the average of 5 measurements on each microsite.

NDVI saturated at high LAI (LAI >2) reaching a maximum of 0.86 in the watered treatment where the highest LAI was also measured (4.4 m^2 m^{-2}). In 2010 LAI showed a 7-fold variation across the experiment, from 0.46 m^2 m^{-2} in the drought plot to 3.48 m^2 m^{-2} in the watered plot (see figure 2 in Chapter 5 for a representation of a typical sward on the experiment). Similarly, in 2011 a 12-fold variation was observed in LAI, from 0.33 m^2 m^{-2} in the drought plot to 4.4 m^2 m^{-2} in the watered treatment. Compared to the controls, for the entire data set, LAI was 30% lower in the drought plot and 30% higher in the watered plots and significantly different ($p<0.05$ simple contrasts in nlme). But for each year considered independently LAI was only weakly significantly different from control in the drought plot ($p<0.1$) (Table 1). LAI was was 26% lower in the shallow soils when compared with the deeper ones ($p<0.01$ simple contrasts in nlme).

Ecosystem CO_2 exchange

The response of GPP to light showed a saturating pattern across the experiment, although differences in the response (Figure 1) were evident between treatments, between measuring days and between soil depth categories within treatments. Overall an average relative mean square error (RMSE) of 1.18 μmol CO m^{-2} s^{-1} (±0.07 [standard error]) was estimated for the prediction of all light curves.

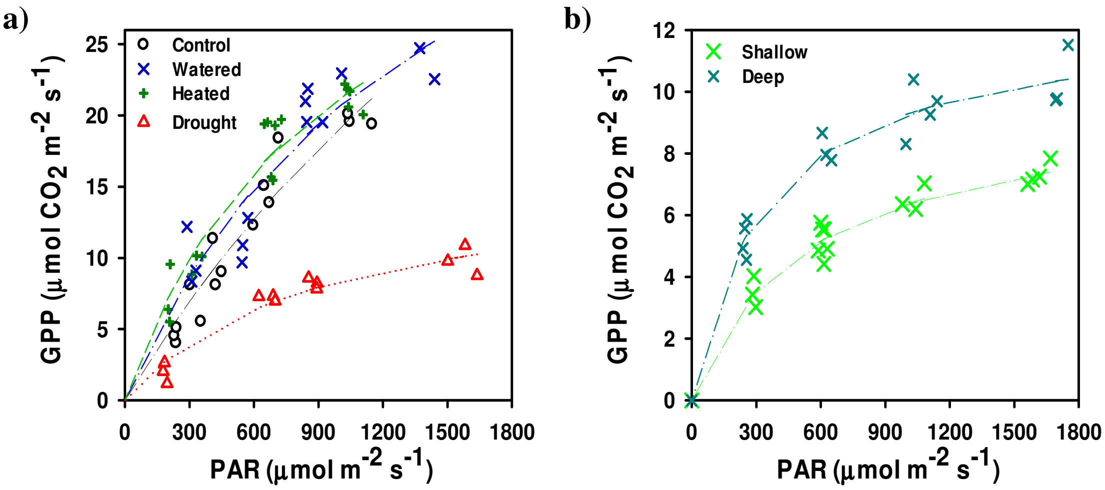

Figure 1. Response of gross primary productivity (GPP) to photosynthetic active radiation (PAR), illustrating among and within-treatment variation. **a)** Microsites across different treatments in Block A on deep soils. All fluxes obtained in 2011: 29th of July (control), 30th of July (watered), and 2nd of August (heated and drought). **b)** Microsites in the heated treatment in Block D on shallow and deep soils obtained on the 17th of July 2011. Note the different y-axis scale on the right panel.

The fitted parameters for each light curve showed a large range of variation; E_o, the initial slope of the light response curve, varied between 0.01 and 0.11 (μmol CO_2 μmol^{-1} PAR), E_o showed a significant correlation with soil moisture ($r=0.381$ $p<0.001$, $n=81$) and NDVI ($r=0.390$ $p<0.001$, $n=81$). Average value of $P_{max,}$ the rate of light saturated photosynthesis, was 29.18 ± 2.24 μmol CO_2 m^{-2} s^{-1} and it was correlated to LAI ($r=0.289$ $p<0.01$, $n=81$) but not to any of the environmental drivers (soil properties or air temperature). Average light compensation point, LCP, estimated from the previous parameters was 276.9 ± 15.7 μmol PAR m^{-2} s^{-1}, but it presented up to a 10 fold variation between the lower and higher values, which is likely related to the high variability in LAI present in the experiment. LCP was significantly correlated to air temperature ($r=0.404$ $p<0.01$, $n=81$).

Light-adjusted GPP and Ecosystem Respiration

GPP estimated at 600 μmol m^{-2} s^{-1} of PAR (GPP_{600}) and temperature-adjusted ecosystem respiration (R_{eco15s}) experienced high within-treatment variability (Figure 2). However, the level of variability was similar for the control, the drought and the watered treatment in each year and across the entire data set (CV% between 29-33%), whilst the R_{eco15s} presented a larger range of variation (34 to 49% CV). The variability observed for the heated treatment was similar for both fluxes (CV=45%).

Across both measurement years, the lowest values for GPP_{600} were observed in the drought treatment and the highest in the watered treatment. In 2010, compared to the control, GPP_{600} was 30% lower in the drought treatment (6.48 ± 0.49 μmol CO_2 m^{-2} s^{-1}) and 38% higher in the watered treatment (13.52 ± 1.15 μmol CO_2 m^{-2} s^{-1}); and both were significantly different from the control (Figure 2). In 2011, the lowest GPP_{600} was measured in the drought treatment (7.73 ± 0.73 μmol CO_2 m^{-2} s^{-1}), approximately 30% lower than the control; and the highest in the watered treatment (13.23 ± 1.20 μmol CO_2 m^{-2} s^{-1}), but only the drought treatment was different from the control (Figure 2). The heated treatment was not significantly different from the control in 2011, the only year when it was evaluated.

Results for $Reco_{15s}$ (Figure 2) presented a similar pattern to the observed for GPP_{600}. $Reco_{15s}$ was 65% larger, than the control, in the watered plot on 2010 (9.80 ± 1.26 μmol CO_2 m^{-2} s^{-1}) and the drought was 20% lower (4.06 ± 0.27 μmol CO_2 m^{-2} s^{-1}), although only the former was significantly different from the control. In 2011, the drought treatment was the only significantly different from the control (50% lower, average 4.94 ± 0.64 μmol CO_2 m^{-2} s^{-1}).

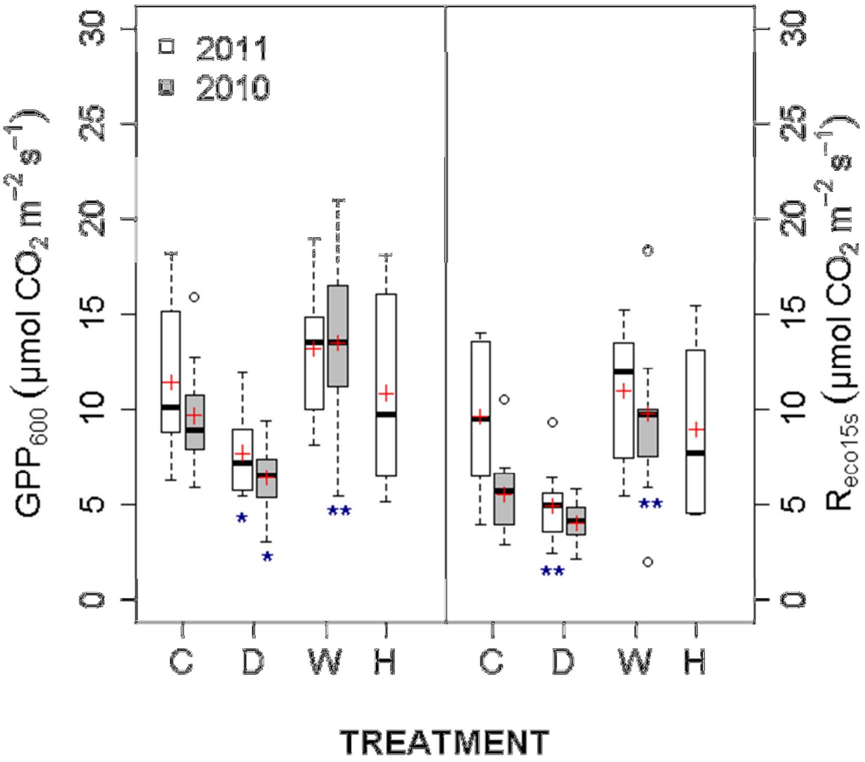

Figure 2. Variability of the CO_2 fluxes measured at Buxton during 2010 and 2011. GPP_{600} refers to the productivity estimated at 600 µmol PAR m^{-2} s^{-1} and R_{eco15s} is the ecosystem respiration standardized across the experiment using soil temperature (at 15 °C). Each box displays the median, upper and lower quartiles of the respective distribution. Box whiskers represent the maximum and minimum range of the data. Means of the distribution are represented by red crosses. n=14 per treatment in 2010 and n=10 in 2011. C=control, D=drought, W=watered, H=heated (only measured in 2010). Boxes with a blue asterisk (*) were marginally different ($p<0.1$) or significantly different (**,$p<0.05$) compared to the control on the respective year (simple contrasts in nlme). See Table S1 in Chapter 3b for average values and standard errors.

When the data set was considered for the two years together, the difference in GPP_{600} between the drought plot and the control turned more significant (30% lower, $p<0.05$, simple contrasts in lme) and a signficant different was also observed for the watered plot (30% higher, $p<0.05$, simple contrasts in lme). In terms of $Reco_{s15}$ the difference in the entire data set was highly significant ($p<0.01$, simple contrasts in lme) for both the drought (40% lower) and the watered (34% higher) treatments. Within-treatment variation of the fluxes from 2010 to 2011 was only significant for

R_{eco15s} in the control plots, where the average for 2011 (9.61 ± 1.24 µmol CO_2 m^{-2} s^{-1}) was 60% higher than in 2010.

Contrary to expectations no significant within-treatment difference was observed for the CO_2 fluxes between soil categories. However in the heated plots GPP_{600} was up to 30% lower at the shallow microsites, but these differences was not statistically significant.

GPP_{600} and R_{eco} relationship

As expected GPP_{600} and ecosystem respiration (R_{eco}) were strongly coupled in 2010 and 2011 (Figure 3) and when both data sets were considered together (R^2=0.64, p<0.001, $F_{1,79}$=144.1, slope=0.79). There were no significant differences in the GPP_{600}-R_{eco} slopes between treatments (within each year) (Table S2 Chapter 3b), what suggests no-decoupling of the relationship caused by the treatments. The GPP_{600}-R_{eco} at the watered treatment was not-significant in 2011. When the relationship was considered for the entire data set, a significant difference between years (slopes) was observed (Figure 3).

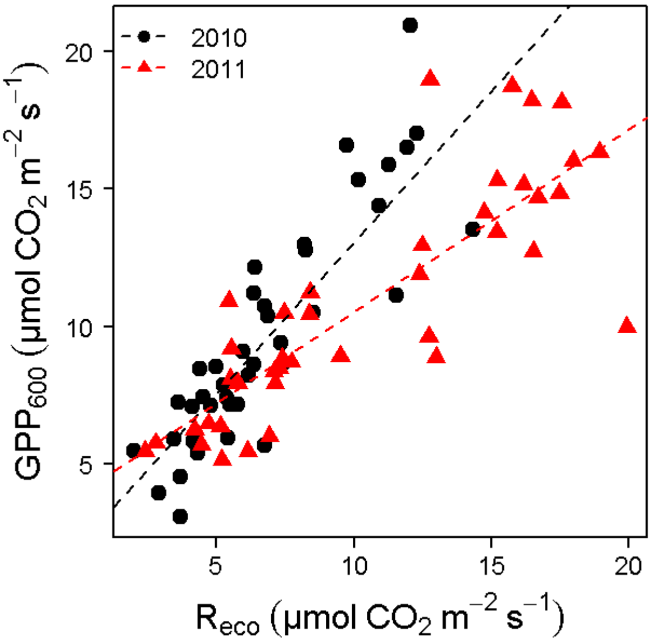

Figure 3. Relationship between Gross primary productivity at 600 µmol PAR m^{-2} s^{-1} (GPP_{600}) and Ecosystem Respiration (R_{eco}). **2010**: β=1.10, R^2=0.73, p<0.001, n=41, $F_{1,39}$=109.5. **2011**: β=0.66, R^2=0.68, p<0.001, $F_{1,38}$=87.26, n=40. Slopes were significantly different (p<0.001).

Drivers of variation in GPP_{600}

GPP_{600} variation across the experiment was largely influenced by variations in soil moisture and to a less extent by variations in leaf area index (LAI). We found a significant but weak linear relationship between GPP_{600} and LAI (R^2=0.09, $F_{1,79}$= 8.9, p<0.01, RMSE=3.9). Our data also suggests a non-linear relationship with a saturation for LAI>3 (see Figure 4), as previously reported for high LAI (Chapin III *et al.*, 2002); however the use of an asymptotic function (Michaelis-Menten) model to explored the relationship between GPP_{600} and LAI did not improved the RMSE significantly (change < 2%). Soil moisture on the other hand, was an important driver of GPP_{600} (R^2=0.26, $F_{1,79}$=30.0, p<0.001, RMSE=3.05), and it also presented a saturation response to LAI and to GPP_{600} (Figure 4).

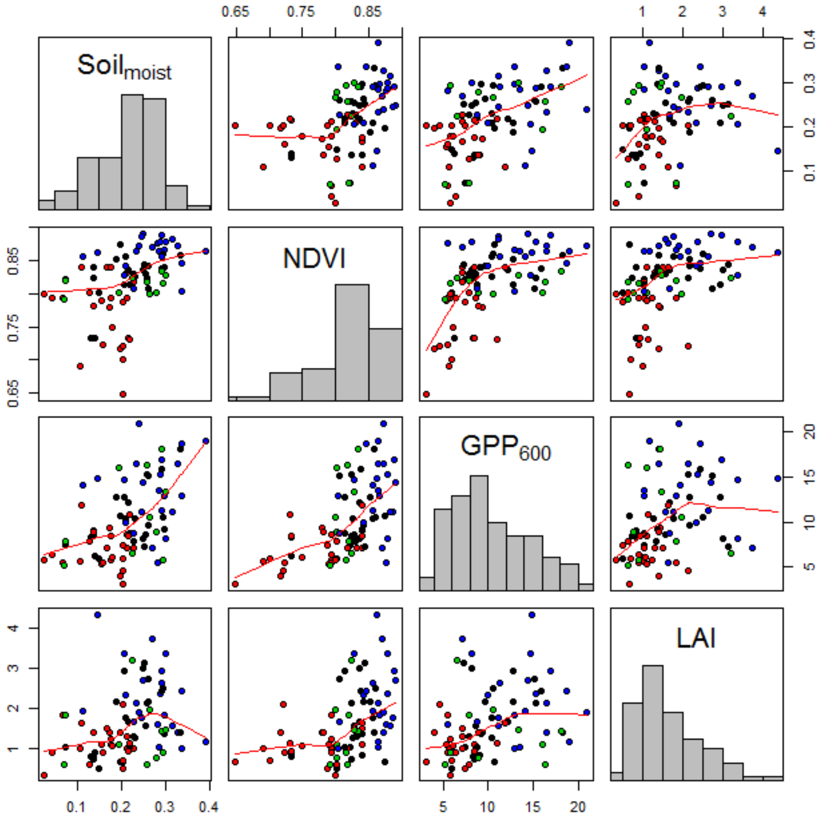

Figure 4.Frequency distribution of the biomass properties (NDVI and LAI [m^2 m^{-2}]), soil moisture and the CO_2 fluxes for the entire data set. Red lines are lowess curves (locally weighted regression) for the pair of variables. All variables are significantly correlated at p<0.01 (see table 2 for details). Treatments are represented by colours: Control=black, Drought=red, Heated=green, Watered=blue).

Soil moisture and LAI together were able to explain approximately 35% of the variance observed on GPP_{600} (Table 3). And the use of the mixed effect model reduced RMSE from 3.25 μmol CO_2 m^{-2} s^{-1} (in the linear model approach) to 2.70 μmol CO_2 m^{-2} s^{-1}.

Table 3. Estimated parameters for the relationship between leaf area index (LAI), soil moisture and GPP_{600}.We present the parameters for both the linear model and the mixed effect model. n=81. A term to account for the curvature between soil moisture and LAI was included (Soil Moisture^2).

Variable	**Linear Model**		**Mixed-effect model**	
	Coefficient	**SE**	**Coefficient**	**SE**
Intercept	3.98	2.68	6.34**	2.31
Soil Moisture^2	140.76**	54.1	99.21*	55.02
Soil Moisture	-11.91	22.5	-12	23.2
LAI	4.119**	1.634	0.92*	0.47
LAI:Soil Moisture	-14.061*	7.105	NS	
Model statistics:	R^2= 0.35,	AIC=433	[1]R^2= 0.49 (0.36),	AIC=420
	RMSE=3.25	BIC=447	RMSE=2.70	BIC=439

*p<0.1,**p<0.05,***p<0.001. [1]R^2 values are conditional (variance explained by both fixed and random factors [Year/Block/Plot]) and marginal the variance explained by the fixed factors only (in parenthesis).

Species composition and their role on GPP_{600} and R_{eco}

Our results for the nonmetric multidimensional scaling (NMDS) analysis for the microsites in 2011 (Figure S1 Chapter 3b), showed a segregation of the samples associated to the drought treatment from the controls. However, samples were in general associated with different values of axis 1 and 2 so no clear specific species dominance was evident. From the regression models evaluated, the contribution of the species composition was dominated by the first 2 axes (so the third axis was excluded from the model). The species composition explained 34% of the variance in $Reco_{15s}$ ($F_{2,36}$ =10.84, p<0.001) and 27% in GPP_{600} ($F_{2,36}$ =7.87, p<0.01).

Community leaf nitrogen content and Total Foliar Nitrogen

Although our results showed that the fluxes are largely determined by soil moisture and LAI (Table 3), including the community leaf nitrogen content (LNC_{agg}) and its significant interaction with LAI explained an additional 9% of the observed variability on GPP_{600} with a 5% reduction on the RMSE (Table 4). A similar increment on the determination coefficient (R^2) and a reduction on RMSE were observed on the non-adjusted R_{eco}, however the fixed factors showed a less significant contribution. When LNC_{agg} was considered alone or in combination only with soil moisture its contribution was not significant.

Table 4. Description of the models used to assess the contribution of LNC_{agg} to GPP_{600} and R_{eco} (adjusted and non-adjusted), Akaike's information criteria (AIC), Bayes Information Criterion (BIC), loglikelihood (LogLik), variance explained (R^2) and significance (p). Models were fitted using Soil Moisture (SM) and Leaf Area index (LAI) only, or additionally including Community Leaf Nitrogen content (LNC) and the interaction with LAI. Only for microsites studied in Buxton during 2011.

Response variable	**Parameters**	**Model evaluation**					
		AIC	**BIC**	**LogLik**	**RMSE (μmol CO_2 m^{-2} s^{-1})**	**$^{!}R^2$**	**$^{!!}p$**
GPP_{600}	SM***, LAI**, LNC*, LAI:LNC**	210.5	222.9	-97.24	2.87	0.50 (0.46)	<0.001
	SM, LAI	212.0	222.3	-110.31	3.03	0.46 (0.37)	<0.001
R_{eco}	SM***,LAI*, LNC, LAI:LNC*	224.7	237.1	-104.33	3.20	0.57 (0.31)	<0.001
	SM**, LAI	226.3	236.0	-107.14	3.32	0.58 (0.21)	<0.001
R_{eco15s}	SM***,LAI, LNC, LAI:LNC*	205.3	217.8	-94.33	2.43	0.62 (0.38)	<0.003
	SM***, LAI	205.9	215.5	-96.94	2.54	0.61 (0.34)	<0.002

Significance of the parameters: *p<0.1,**p<0.05,***p<0.001. $^{!}$ R^2 values are conditional (variance explained by both fixed and random factors) and marginal (variance explained by the fixed factors only), in parenthesis. $^{!!}$Significance of the model (p) is estimated from the comparison of the nested models (this model with only the random factors). The models were fitted with Block/Plot as random factors.

TFN estimated from measurements of indirect LAI and LNC_{agg} using the harvest data from 2010 (30 samples) presented a strong relationship with the TFN estimated from direct harvest the same year (R^2=0.54, p<0.001, RMSE: 0.61 g N m^{-2}). This relationship is summarised in Equation 4:

$$TFN = -1.463 + 0.493 * LAI + 0.249 * LNC_{agg} \qquad \text{Equation 4}$$

Where LNC_{agg} is the weighted mean for the microsite estimated with Equation 3 using biomass relative abundance as the weighting factor, and LAI is the indirect measurement with the LAI-2000. And where TFN is estimated was estimated as described in Chapter 2 ($TFN = \sum_{i=1}^{n} LNC_i \times LAI_i$ where i represents each species).

As it was not feasible to harvest biomass in the microsites under study we used Equation 4 to estimate TFN for all microsites in 2011 and for microsites only in the control plots in 2010. Our results of TFN, estimated in this manner, showed an important variation of TFN for the treatments in 2011 ranging from 2.37 to 4.84 g N m^{-2}. However, the drought treatment was the only significantly different from the control ($p<0.05$ Pair-wise comparison, Table S3 Chapter 3b). TFN showed a significant relationship with the fluxes in 2010 in 2011 in the control plots. Although no major differences were observed in the TFN-GPP_{600} relationship ($R^2=0.17$, $F_{1,22}=5.66$, $p<0.05$), in comparison with the ecosystem respiration: R_{eco} ($R^2=0.16$, $F_{1,22}=5.35$, $p<0.05$), and R_{eco15s} ($R^2=0.15$, $F_{1,22}=5.11$, $p<0.05$). However it was clear that the relationship between the two variables was affected by the year when they were estimated (Figure S2 Chapter 3b), particularly for respiration, and that the R^2 was greatly improved if the data were fitted with independent slopes.

Discussion

Treatment effects on the fluxes and the relevance of the weather conditions in the preceding growing season

Our results support the hypothesis (H1.1) that the productivity of the ecosystem is lower when the conditions are more water-limited. Compared to the control, there was a significant decline in GPP_{600} values observed in the drought plots, and 30% increase in the watered plots (Figure 2). Additionally, our results suggest that the response to the imposed treatments is largely the combination of the conditions during the measurement period (i.e. soil moisture, temperature, radiation) and the effect off conditions experienced during the preceding part of the current growing season. These effects include the development of the leaf area and the activity and development of the associated heterotrophic communities (Staddon *et al.*, 2003, Bardgett and Wardle, 2012).

We found a strong coupling between GPP_{600} and R_{eco}, further supporting (H1.1), but the slope of the relationship was significantly different between years (Figure 3). This difference is likely associated to the relative increment of R_{eco} in 2011 (significant for the control plots), which is likely associated with the measurement conditions experienced during this year (i.e. higher PAR, and its control on soil temperature), and higher soil moisture. These conditions might produce a faster transference of assimilates in the ecosystem, through a direct impact on root exudation and stimulation of microbial activity (Bardgett *et al.*, 2013).

The relative differences observed in the fluxes between years could also be a result of the dry spring experienced in 2010 (approximately 43% less rain than in 2011). The drought might have affected other components of the ecosystem, like bryophytes (that could represent up to 15 % dry aboveground biomass as recorded in the separate harvest on 2010), and that have been shown to be largely controlled by moisture on this experiment (Bates *et al.*, 2005). The possible effect of the dry spring on this component could also explain why we observed differences in the fluxes between years, despite similarities in LAI, as the bryophyte component could contribute to a significant fraction of the of the ecosystem productivity (Street *et al.*, 2007, Douma *et al.*, 2007), but it can hardly be accounted for in terms of LAI when indirect methods of estimation (i.e. LAI-2000) are used.

Soil-biomass interaction on the control of the CO_2 fluxes

We did not find any significant difference in fluxes with soil depth per se, which partially falsifies the second statement on hypothesis (H1.1) about the role of soil depth on the fluxes. However, our results suggest that there is an interaction between soil depth, soil moisture and above-ground biomass (LAI, NDVI and vegetation height) (Figure 4, Table 2) in the control of the fluxes (supporting H2.1), but these drivers certainly work at difference scales.

Soil depth probably has a major role in the development of belowground structures that are important to sustain LAI. The above ground biomass LAI was 26% lower in shallow soils, and it is likely that areas with deeper soils have deeper rooting systems, which could be associated to higher LAI. However the lack of a more direct effect of soil depth on the CO_2 fluxes could be further explain by two possible mechanisms. First, due to the limited water holding capacity of the soils at the site (Grime *et al.*, 2008), it is likely that species in shallow soils have developed root systems that allow for the exploration of fissures present in the soils, therefore keeping productivity despite limiting conditions. Second, the presence of species with an important lateral spread, i.e. *Carex* spp*, Campanula rotundifolia, Koeleria macrantha, Agrostis* spp. among others (Bilton *et al.*, 2010, Whitlock *et al.*, 2007, Grime *et al.*, 2007), would mean access to resources outside our 0.4 m^2 measurement area.

It is likely that soil moisture's effect on the fluxes is through the control of the heterotrophic communities, and perhaps through the indirect control of soil temperature. Our results (Table 2) are not surprising, particularly considering that the main effect of the treatments is on the manipulation of the available water (Risch and Frank, 2007, Dagg and Lafleur, 2011). The control that soil moisture could have on the heterotrophic respiration is perhaps one the reasons why the ecosystem respiration was reduced in 2010.

Contrary to findings elsewhere (Frank, 2004, Campioli *et al.*, 2009, Shaver *et al.*, 2007, Street *et al.*, 2007), we did not find a strong relationship between LAI and GPP_{600}. However, LAI in combination with soil moisture were able to explain 35% of the variance observed on GPP_{600} (Table 3). The lack of a strong relationship between LAI and GPP_{600} ($R^2<0.1$) could be caused by the saturation of the LAI-GPP relationship (Figure 4) observed elsewhere for high LAI values (Otieno *et al.*, 2009, Byrne *et al.*, 2005), or by the underestimation of LAI when it is measured with the LAI-2000 (see Chapter 2 where LAI measured during 2010 had a maximum of 4.1 $m^2\ m^{-2}$ while the harvest reached up to 7.9 $m^2\ m^{-2}$). Additionally, the weak LAI-GPP relationship could be explained by the over shading of active leaves by relative inactive leaves, what causes sub-optimality in canopy photosynthesis (Fletcher *et al.*, 2012).

The role of species composition and their role on available N

The results of this study indicate that up to 35% of the variability in the CO_2 fluxes (35% for R_{eco15s} and 25% for GPP_{600}) can be explained by the species composition, which supports our hypothesis (H3.1). Although we also explored the relationship of the fluxes with other common measures of species composition (diversity, richness), we did not find any significant relationship (Table S3 Chapter 3b), despite suggestions that species richness might play an essential role on the buffering of climate change (Saleska *et al.*, 2002).

Our results also show that the role of species composition and their traits is relevant in the context of their abundance and their quality (Hirota *et al.*, 2010). This was evidenced by the significant LNC-LAI interaction (Table 4) and the important increment in the explained variability of GPP_{600}. However this contribution seemed to be more important for GPP_{600} than for R_{eco} (Table 3), perhaps because R_{eco} in the short term is largely influenced by soil moisture. The relevance of species abundance (i.e. biomass) also explains why our TFN estimations (Figure S2 Chapter 3b), were similar for the two years, as LAI in the control plots did not change significantly from 2010 to 2011.

Relationship between instantaneous GPP and annual NPP

The temporal framework of this work only allowed us to assess the effect of the treatments on the instantaneous fluxes during the peak growing season, when they were characterized by a large variability (Figure 2). However, it is possible to identify potential mechanisms to explain why, despite the significant differences observed in the treatments (particularly in the watered treatment), the NPP, evaluated as harvested aboveground biomass at the end of the season, remains fairly constant.

Our results for the drought plot suggest that the response of the treatment is the result of the significant reduction in leaf area index, which is probably caused by a higher relative allocation to belowground structures, which allows plants to explore deeper water resources. However, an additional reduction on ecosystem GPP might be caused by a lower activity of the heterotrophic communities (particularly in 2010) under the water-limited conditions as it limits decomposition and nutrient cycling. However, some level of recovery of the ecosystem is expected after the drought through the reported increment in soil N at the end of the treatment (Fridley *et al.*, 2011), that could minimize the observed differences later in the year.

The response on the water treatment is likely the combination of higher LAI, higher respiration but also a faster incorporation of litter into the soil. This treatment presented the highest LAI (3.48 m^2 m^{-2} in 2010 and 4.4 m^2 m^{-2} in 2011). The high values of LAI and the saturation of the response of GPP to LAI (Figure 4), would suggest that leaf shedding and rapid replacement of leaf area is essential to maintain a high level of photosynthesis on this treatment. Although this faster leaf turnover would be reflected in a higher amount of litter, our direct harvest carried out in 2010 (when the watered treatment was higher than the control) suggested that a lower amount of litter was present on this treatment (at least 20% lower than control and other treatments). The difference in litterfall was not statistically significant (Chapter 2), but considering that the species at this treatment have lower LMA we could suggest that a faster decomposition of materials and incorporation into the soil is likely, what could change heterotrophic activity and ecosystem respiration. However, as we measured only one month after the start of the treatment, we do not know if irrigation causes just a short term benefit that is lost later in the season, or if the ecosystem is so adapted to pulses of water/nutrients that the relative differences on spring-rainfall had no major effect on the ecosystem productivity during the summer.

At least two possible mechanisms could be suggested to explain why there was no difference in the CO_2 fluxes between the heated treatment and the control. First, the advance in the growing season (Grime *et al.*, 2000), might only shift the time when the ecosystem becomes a sink with a null effect on the integrated annual budget of CO_2 (Saleska *et al.*, 1999). Second, it has been suggested that the effect of artificial warming on ecosystem productivity depends largely on which factor is most limiting to plant growth (De Boeck *et al.*, 2007, Harper *et al.*, 2005). Thus, considering the limited water-holding capacity of the soils on the site, that GPP_{600} was 30% lower in shallow soils, and that LAI was not higher than in the control, we could suggest that the heated treatment creates drought stress in shallow soils, that could perhaps change patterns of carbon allocation towards underground structures, with lower allocation to LAI affecting GPP and NPP. However as this effect it is likely limited to shallow soils the overall effect of the treatment might be less significant when deeper soils are considered.

Conclusions

The treatments have an important effect on GPP and $R_{eco,}$ particularly the watered and the drought, but our results show that the fluxes are also affected by a combination of abiotic factors and aboveground properties (biomass). Soil depth affects rooting depth, while soil moisture has a more direct effect on the respiratory fluxes by affecting heterotrophic activity and soil temperature. The amount of aboveground biomass determine the photosynthetic area of the ecosystem but also plays an important role in the moderation of external forcing affecting soil temperature in particular (Klein *et al.*, 2005).

The inter-annual variability observed in the control (R_{eco15s} 60% higher in 2011), but not in the treatments, suggest that the observed responses might also be a result of the control plots reacting differently to inter-annual variability in ambient conditions. This would certainly affect the estimated differences between treatments and control, and this has been suggested as one of the main complications of long-term experiments of this kind (Beier *et al.*, 2012).

The species present on the different treatments have developed properties that allowed them to maximize productivity under the given conditions, but it is the combination of these traits with their abundance what controls their role on GPP. Species abundance and their leaf traits determine the quality and quantity of the litter, and the available total foliar nitrogen, which has a strong relationship with both R_{eco} and GPP. Although our results for TFN are lower than reported by direct harvest, probably by the lower LAI estimated with the LAI-2000, and that percent cover surveys usually miss an important number of species (Borcard *et al.*, 2011), our estimations for LNC_{agg} are in agreement with values previously obtained for the site (Chapter 2),which provides further confidence on the approach used here.

Despite the temporal limitation of our study, the non-destructive approach used here captured the small scale spatial variations on CO_2 fluxes, and allowed for a more detailed exploration of the link between ecosystem's CO_2 fluxes, biomass and species composition. Undoubtedly our understanding of the controls of primary productivity on these grassland could be largely improved by the integration of our measurements over a longer period of time (through direct measurements or modelling), by the isolation of the respiratory fluxes (autotrophic and heterotrophic), and by the consideration of the extent to which plant-soil interactions feedbacks are driven by climate versus soil fertility (Bardgett *et al.*, 2013).

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Chapter 3b. Soil moisture, leaf area index and inter-annual climatic variability control gross primary productivity in limestone grasslands in a field-scale climate manipulation experiment-Supplementary material

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Light-adjusted GPP and Ecosystem Respiration

Table S 1. Mean values of the CO_2 fluxes measured at Buxton during 2010 and 2011. GPP_{600} refers to the productivity estimated at 600 PAR (μ mol PAR m^{-2} s^{-1}) and R_{eco15s} stands for the ecosystem respiration standardized across the experiment using soil temperature. Values in parenthesis are standard error for the measurements. n=14 per treatment in 2010 and n=10 in 2011. The heated treatment was not included in the 2010 data set.

Treatment	**2010**				**2011**			
	GPP_{600} (μmolm^{-2} s^{-1})		R_{eco15s} (μmolm^{-2} s^{-1})		GPP_{600} (μmolm^{-2} s^{-1})		R_{eco15s} (μmolm^{-2} s^{-1})	
Control	9.68		5.51		11.42		9.61	
	(0.70)		(0.53)		(1.20)		(1.24)	
Drought	6.48	*	4.06		7.73	*	4.94	**
	(0.49)		(0.27)		(0.73)		(0.64)	
Watered	13.52	**	9.80	**	13.23		11.0	
	(1.15)		(1.26)		(1.20)		(1.14)	
Heated	-		-		10.86		8.96	
					(1.52)		(1.37)	

*Treatment was marginally different ($p<0.1$, simple contrasts in nlme) or ** significantly different from the control ($p<0.05$, simple contrasts in nlme).

GPP_{600} and R_{eco} relationship

To evaluate if the treatment had a decoupling effect on the GPP_{600}-R_{eco} we analysed the data independently by treatment on each year (Table S 2). No significant difference was observed between slopes on 2010 or 2011(the watered treatment was excluded from this comparison as the GPP_{600}-Reco was not significant in 2011).

Table S 2. Regression analysis between GPP_{600} and ecosystem respiration. Summary statistics included: Slope and standard deviation (in parentheses), Intercept, significance value (p), coefficient of determination (R^2), F ratio (F) and number of samples (n). No significant difference was observed between treatments on each year (slopes were compared with analysis of covariance-ANCOVA-)

Year	**Treatment**	**Slope**	**Intercept**	**p**	**R^2**	**F**	**n**
2010	Control	1.13 (0.02)	2.45	<0.001	0.78	49.0	14
	Drought	0.96 (0.30)	1.63	<0.01	0.42	10.3	14
	Watered	0.82 (0.24)	5.32	<0.01	0.47	11.5	13
2011	Control	0.67(0.13)	3.98	<0.001	0.75	28.0	10
	Drought	0.55(0.20)	4.17	<0.05	0.44	8.1	10
	Watered	0.39(0.25)	8.09	N.S	0.13	2.4	10
	Heated	0.75(0.09)	2.44	<0.001	0.86	57.9	10

N.S. non-significant.

Species Richness

We also evaluated the contribution of species richness, functional group richness and diversity (Simpson Index), but no significant relationship was found with the CO_2 fluxes. Overall for the data examined in 2011 species richness and average functional group were lower in the drought treatment and higher and significantly different in the watered treatment (Table S3). Diversity evaluated with the Simpson Index (Table S3) was only significantly lower in the drought plot which is probably associated to a lower number of species.

Table S3. Mean values of Simpson diversity index, species richness, community leaf nitrogen content (LNC_{agg}), average number of Functional groups and Total Foliar Nitrogen (TFN) for the microsites studied in Buxton during 2011. Values in parenthesis are standard error for the measurements. n=40 (10 per treatment).

Variable	**Average**	**Coefficient of Variation (%)**	**Range (Minimum-Maximum)**	**Significantly different from control***
Diversity (SI)	0.89 (0.01)	4.09	0.75-0.94	Drought
Species Richness	12 (1)	27.17	6-18	Drought Watered
LNC_{agg} (mgN g^{-1})	15.4 (0.2)	7.35	13.0-17.5	Heated Watered
Functional groups	3.54 (0.16)	29.35	1.75-5.68	Drought Watered
TFN (g N m^{-2})	3.19 (0.09)	17.4	2.37-4.84	Drought

*Treatment was significantly different from the control in pairwise comparison with Bonferroni adjustment (p<0.005).

Species composition analysis

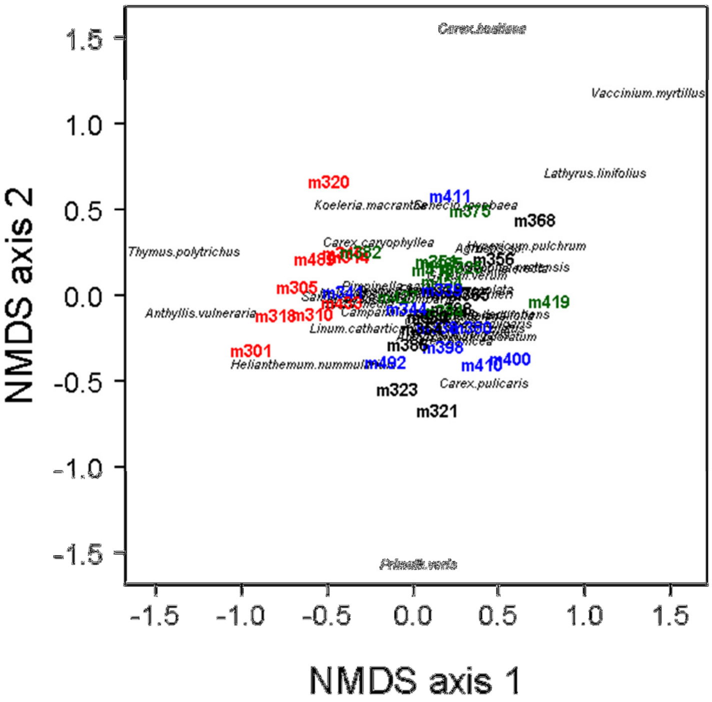

Figure S 1. Compositional relationship of microsites evaluated on 2011 as shown by a two-axis nonmetric multidimensional scaling (NMDS) ordination. Numbers represent each individual microsite in the Drought (red), Watered (Blue), Control (Black) and Heated (Green) treatments.

TFN and CO_2 fluxes relationship for 2011 data set

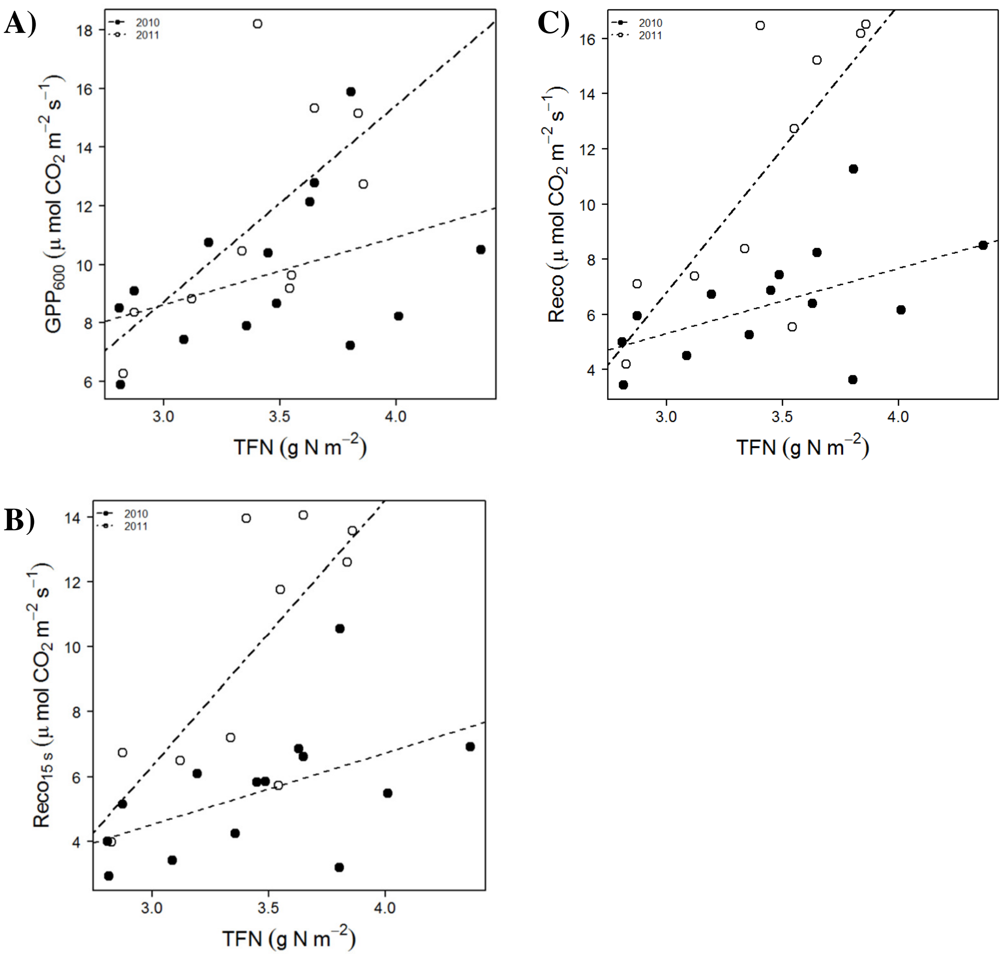

Figure S2. A) Relationship between GPP_{600} and Total foliar Nitrogen (TFN) for control plots. **2010**: $\beta=2.30$, $R^2=0.09$, non-significant, **2011:** $\beta=6.67$, $R^2=0.34$, $p<0.05$. **B)** Relationship between R_{eco} and TFN. **2010**: $\beta=2.359$, $R^2=0.22$, $p<0.05$.**2011:** $\beta=10.43$, $R^2=0.54$, $p<0.01$. Slopes were significantly different. **C)** Relationship between $Reco_{15s}$ and TFN for control plots. **2010**: $\beta=2.21$, $R^2=0.21$, $p<0.1$. **2011:** $\beta=8.18$, $R^2=0.53$, $p<0.01$.Slopes were significantly different. All relationships are for control plots only with n=14 in 2010 and n=10 in 2011.

Chapter 4a. Spatial and seasonal variability of photosynthetic parameters and leaf traits in a Neotropical seasonally dry forest

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Abstract

To improve our understanding of the limitations to productivity in the seasonally dry tropical forest (SDTF) we estimated two photosynthetic efficiency parameters, commonly used for ecosystem modelling purposes (maximum RuBP saturated rate of carboxylation, V_{cmax}, and electron transport driving regeneration of RuBP, J_{max}). We explored the relationship between these parameters and nitrogen (N) and phosphorus (P) content at leaf level, seasonally and across a successional gradient. We selected four dominant species along a post-disturbances chronosequence (15, 25, 65 and >100 years) in the SDTF of Costa Rica, and we carried out our measurements during the rainy, the transition from rainy to dry season and in the early dry season. Our estimations of V_{cmax} (26.6-175.7 µmol CO_2 m^{-2} s^{-1}) and J_{max} (47.4-205.1 µmol CO_2 m^{-2} s^{-1}, measured only in the rainy season) are consistent with reports for other water limited ecosystems. None of these parameters showed significant variation along the chronosequence, and V_{cmax} did not show a significant seasonal variation at site level. During the rainy season, V_{cmax} was strongly related to N (R^2=0.40) and P (R^2=0.45), however the role of P was not observed later in the year, as P was probably remobilized. Similarly, J_{max} showed a correlation with N and P during the rainy season (R^2=0.48 and 0.52 respectively). Along the chronosequence the bivariate relationships, explored in the rainy season only, showed no major differences in the slopes between the 25 and 65 years stands, but the relationship decoupled at the extremes of the chronosequence probably due to high intra/inter-specific variability. Additionally, we estimated the photosynthetic rate at saturating conditions (A_{sat}), and found that stomatal conductance was the main controlling factor, but an important relationship with N and P was also observed. Finally, we explored within-species traits variation for four species across the chronosequence, and we found that structural traits were more variable both seasonally and spatially than the photosynthetic parameters, but that the patterns of variation were consistent with results at plot level.

Introduction

The seasonally dry tropical forest (SDTF) represents approximately 700 000 km^2 in the Neotropics (Linares-Palomino *et al.*, 2011), mostly in areas that are recovering from human disturbance. The SDTF is characterised by a strong rain-seasonality (Chapotin *et al.*, 2006b, Bullock *et al.*, 1995), that along with soil's physical and topographic heterogeneity define species composition (Bullock *et al.*, 1995, Leiva *et al.*, 2009b), and most of the plant's physiological and phenological processes (Rojas-Jimenez *et al.*, 2007, Holbrook *et al.*, 1995).

Due to the central role of rain-seasonality on this ecosystem, it is not surprising that most of the research addressing limitations to productivity (photosynthesis), has predominantly focused on the control that dynamic changes in water availability exert on gas exchange (Brodribb and Holbrook, 2007, Brodribb *et al.*, 2002). So far, several studies have addressed the different strategies in water-use efficiency for species of contrasting phenological habits (Rojas-Jimenez *et al.*, 2007, Villalobos-Vega, 2001), the role of environmental factors on stomatal closure and leaf shedding (Gutierrez-Soto *et al.*, 2008), the importance of stem water storage for phenological processes (Chapotin *et al.*, 2006a, b, Borchert, 1994, Fallas-Cedeno *et al.*, 2010), and the strong coordination between hydraulic conductivity and photosynthesis across seasonal transition (Brodribb *et al.*, 2002).

However, far less attention has been paid to how seasonal pulses of nutrients (Quesada *et al.*, 2009, Bullock *et al.*, 1995) might affect the key parameters that determine photosynthesis (but see Kitajima *et al.* (1997a, b), and references in Chaturvedi *et al.* (2011)), or how the photosynthesis-nutrient relationship can be affected by site-specific conditions. Information that is relevant to understand how this ecosystem would respond to seasonal variations in resources. At the SDTF, as in other ecosystems, it is expected that nitrogen (N), the essential component of the ribulose 1.5-biphosphate carboxylase/oxygenase (RuBisCO), will play a central role the control of photosynthesis (Domingues *et al.*, 2010). But it is possible that photosynthesis could also be constrained by phosphorus (P) availability, important in the energy related processes of photosynthesis (van de Weg *et al.*, 2012, Rychter and Rao, 2005), and often highly unavailable in the soils (highly weathered/clayey) where SDTF is often found (Fassbender and Bornemisza, 1994). Additionally, as the co-existence of species with a variety of leaf habits (Powers *et al.*, 2009a, Villalobos-Vega, 2001), and traits for the foraging and use of nutrients and light, is key to maximize the use of resources (Reich, 1995), is likely that the photosynthetic capacity will also be affected by intra-specific and inter-specific variability in the use of resources (Gotsch *et al.*, 2010, Hulshof and Swenson, 2010).

Therefore, in this paper we explore the role of N and P on the seasonal and spatial variation of leaf photosynthetic parameters along a chronosequence in the SDTF in Costa Rica. We focused our work on the estimation of V_{cmax} (the maximum rate of carboxylation), a proxy for photosynthetic capacity related to the in vivo apparent RuBisCO activity, and J_{max} (the electron transport driving regeneration of the ribulose 1.5-bishposphate), parameters that due to its more technical challenging assessment (Long and Bernacchi, 2003) are less frequently included in traits studies. Additionally, we explored the relationship between the photosynthetic capacity traits, their combined expression (photosynthesis at saturating conditions, A_{sat}) and some commonly measured structural traits (leaf mass per area/leaf dry matter content), that provide an indication of carbon allocation and resistance to physical hazard, and are usually correlated with photosynthetic traits (Wright *et al.*, 2004).

The specific questions we addressed on this paper are:

1. Do N and P exert the same control on the variation of V_{cmax}, J_{max} and A_{sat} in sun leaves? Are there clear spatio-temporal patterns on these relationships?

H1.1 We hypothesize that A_{sat} and V_{cmax} will be strongly linked to variations in N, but that variations in J_{max} will more likely be linked to P.

H1.2 Alternatively, we expect seasonal/spatial variation on the photosynthetic parameters-nutrient relationships, associated to shifts in timing of leaf exchange, along the chronosequence, and to seasonality in water availability and vapor pressure deficit (affecting water potential and stomatal conductance).

2. Are individual species showing intra-specific variability in their leaf photosynthetic capacity (A_{sat}, V_{cmax}, J_{max}) and structural/nutritional traits (Leaf mass per area (LMA), P and N content) across the chronosequence?

H2.1 We hypothesize that species traits changes over the chronosequence will match the trait changes observed at plot scale. And that species' photosynthetic capacity will be higher in the early stands of the forest where there is less competition for resources.

H2.2 Alternatively, we hypothesize as the change in species composition (loss of pioneers), define plot traits, and individual species might not follow the same pattern.

The photosynthetic efficiency traits (V_{cmax} and J_{max}) reported here, are not only new for this site but they are also key to improve our understanding of the limitations in the use of resources in this ecosystem. These parameters are also essential input values needed for the up-scaling of ecosystem processes in the SDTF, a step that is needed to understand possible impacts of the forecasted climate change for this area (Enquist, 2002), and its effect on forest physiognomy (Enquist and Enquist, 2011). Additionally, the consideration of the role of P in the control of photosynthesis is novel because given the limited P availability on these soils, species are likely to display different strategies for the use of this resource (Hidaka and Kitayama, 2009), something not yet explored.

Methodology

Site description

The research site is located in the Santa Rosa Sector of the Guanacaste Conservation Area (ACG), in the North Pacific region of Costa Rica (10°30'N, 85°10' W, 280 m.a.s.l). The area is considered part of the Tropical Dry Forest life-zone (*sensu* Holdrige's ecological classification (Tosi, 1969)), and it is an important regeneration zone, where most of the sites are initiated from the introduced grass *Hyparrhenia rufa*, which is still important in the younger stage of the forest.

The area receives on average 1423 mm of rain annually, with a considerable inter-annual variation. A well-defined dry season extends from November to May with a dry spell of one to two weeks in July (Quiros and Stolz, 2010). Soils are of volcanic origin (part of the Ignimbritic series of Liberia (Chiesa *et al.*, 1998)) with an ustic regime (soil is dry at least for 90 or more cumulative days during the year (USDA Soil Survey Staff, 2010)).

Study plots and selected species

Four 1 ha plots (Figure 1) were selected following the floristic description of a chronosequence by Leiva *et al.* (2009a). The plots were set up in April 2010, and the regeneration age (15, 25, 65, >100 years) was established according to the last disturbance event as described in Leiva *et al.* (2009a). We refer here onwards to the study area as a chronosequence, although the assumption that each site had the same initial conditions and has traced the same sequence of changes (Johnson and Miyanishi, 2007, Myster and Malahy, 2008) has not be fully tested. Throughout the text sites are abbreviated as S_{15}, S_{25}, S_{65} and S_{100}, with the number representing the age of the plot.

Figure 1. Sites along a post-disturbance chronosequence, in the seasonally dry forest of Costa Rica. Clockwise from top left corner: early regeneration (15 years, S_{15}), intermediate regeneration (25 years, S_{25}), late regeneration (65 years, S_{65}), mature forest (>100 years, S_{100}). Photos were taken at different times during the year.

Four dominant species (Table 1) were selected on each plot based on a full species inventory included in the study by Leiva *et al.* (2009a). However, in 2011 within each 1 ha plot, a 3000 m^2 area was delimited and all species with a diameter above breast height (DBH) ≥10 cm were identified and measured (DBH and height) to corroborate the selection based on the original inventory. Differences observed between both surveys (Table 1), are likely caused by the location of the 3000 m^2 plot in reference to the 1 ha area, particularly on the early regeneration sites. However, in the smaller survey the species were also dominant.

Table 1. Dominant species selected for the study and soil properties across the chronosequence. Sites are represented by their respective ages since last disturbance (i.e. S_{15}, S_{25}, etc.). Dominant species were selected from basal area data. Values presented are the percentage of dominance within a 3000 m^2 plot and within the 1 ha plot from the inventory in Leiva *et al.* (2009a) (in parentheses).

Species	Family	Phenological habit**	Dominance (%) based on basal area			
			S_{15}	S_{25}	S_{65}	S_{100}
Byrsonima crassifolia	Malpighiaceae	D-LE	35.5 (33.6)			
Cochlospermum vitifolium	Bixaceae	D	4.1 (29.9)	20.5 (27.7)	6.24(3.0)	
Redhera trinervis	Verbenaceae	D	5.1 (16.4)	25.2 (34.5)	24.2 (9.2)	
Semialarium mexicanum	Hippocratea-ceae	E	12.1 (3.4)	10 (1.1)	16.2 (3.8)	
Luehea candida	Tiliaciae	D		3.1 (1.0)	6.2 (10.0)	8.1 (9.1)
Exostema mexicanum	Rubiaceae	D				8.7 (3.0)
Manilkara chicle	Sapotaceae	E				6.1 (12.0)
Hymenaea courbaril	Caesalpinaceae (Fabaceae)	E-LE				12.4 (19.5)
Predominant soil type*			Vertisol/ Entisol	Vertisol/ Entisol	Ultisol/ Entisol	Mollisols

*Dominant soil types as described by Leiva *et al.* (2009b) following taxonomy of USDA-Soil Survey Staff (2010). **E=evergreen, D=deciduous, LE=leaf exchanger or Brevi-deciduos.

Sampling strategy

All measurements were carried out between 2011 and early 2012. Three measuring seasons were established to capture seasonal variation on phenology and resources (water/nutrients): beginning of the rainy season (**RS**, May-June 2011), transition into dry season (**TS**, November-December 2011) and early dry season (**DS**, mid-January to mid-February 2012). All measurements were carried out in the three periods, except for the photosynthetic and leaf traits in *Cochlospermum vitifolium*, as the species had shed its leaves by the DS.

Leaf gas exchange measurements

Gas exchange measurements were carried out using a LI-COR 6400 (LI-COR Lincoln, Nebraska), in at least three different individuals for each dominant species (Table 1). To gain access to the top most part of the tree and to fully sun exposed leaves, branches were detached from the tree and immediately re-cut under water to reconstitute the water column at suggested by Domingues *et al.* (2010).

A/Ci response curves (response of photosynthesis [*A*] to changes in CO_2 concentration) were performed, in fully expanded leaves, within 30-60 minutes after the branch was cut following standard protocols (Long and Bernacchi, 2003). In summary the cuvette was set on the leaf, after stability was reached at CO_2 ~400 µmol CO_2 mol^{-1}, the CO_2 concentration was reduced in steps of 50 µmol CO_2 mol^{-1} until ~25 µmol CO_2 mol^{-1}. Then the CO_2 concentration was set to ~400 until stability was reached and then raised again in steps of 200 µmol CO_2 mol^{-1} until 2000. All measurements were performed under artificial light at 1800 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) provided by the LED light source (Li-COR 6400-02B Red/Blue light source, LI-COR Lincoln, Nebraska). When possible, leaf temperature was controlled throughout the measurement period around 30 ºC (average values and standard deviation for each season: 29.5 ± 0.2 ºC in RS, 31.7 ± 0.3 ºC in TS and 30.9 ± 0.3 ºC in the DS). The rate of photosynthesis under saturating light and CO_2 concentration inside the cuvette of *c.a.* 400 µmol CO_2 mol^{-1}, was extracted from the *A*/Ci curves, and considered as A_{sat}. Diffusion leaks in the leaf chamber gasket of the LI-6400 were corrected with the equation suggested by the manufacturer (LI-COR, 2012), using a CO_2 reference value measured each day before the start of the measurements (average throughout the seasons 407 ± 17 [s.d.] µmol CO_2 m^{-2} s^{-1}).

As it has been suggested that strong alterations of stomatal conductance (g_S) during the development of the *A*/Ci affect the estimation of V_{cmax} (Santiago and Mulkey, 2003), data were excluded when variations of g_S were evident during the development of the curve (26% of the cases, 42 out of 161 curves). An additional quality check suggested elsewhere (Kattge *et al.*, 2009, Domingues *et al.*, 2010, van de Weg *et al.*, 2012) was applied, so curves where A_{sat}/Leaf Nitrogen content < 2 μmol CO_2 g N^{-1} s^{-1} were also excluded (less than 5% of the remaining curves).

Leaf traits

Physical properties

After the gas exchange measurement cycle was finished, the measured leaf was detached from the branch and placed in a plastic bag with moist paper towel, as recommended by Cornelissen *et al.* (2003), and left to re-hydrate for at least 6 hours. To have at least five repetitions, additional leaves were selected from two additional individuals. Once re-hydrated, the leaves were weighted for fresh weight.

Leaves were scanned using a flat-bed scanner and leaf area was estimated with the image J software (Abramoff *et al.*, 2004). After 2-3 days of oven-drying at 60 ºC, leaf dry weight was estimated. Leaf mass per area (LMA, g m^{-2}) and its inverse, Specific Leaf Area (SLA, m^2 g^{-1}) were estimated for each leaf as the ratio between dry weight and leaf area. Leaf dry matter content (LDMC, mg dry mass g^{-1} fresh mass) was calculated as dry weight/fresh weight.

Leaf nutrient content

Nutrient analysis was carried out at the Crew Laboratories at The University of Edinburgh in all the dried leaves. Leaf nitrogen content (N) and leaf carbon content (C, to estimate C:N ratio), were determined using a using a Carlo Erba NA 2500 Elemental Analyser (Carlo Erba Instruments, Milan, Italy). Leaf phosphorus content (P) was analysed with the molybdenum blue method using a Bran & Luebbe Series 3 Autoanalyser (SEAL Analytical, Mequon, Wisconsin, USA). Sample digestion for the P analysis was carried out on 100 mg of oven dried material with a modification of the sulphuric acid-hydrogen peroxide digestion described by Allen (1989).

Leaf traits expression

The use of leaf photosynthetic traits expressed on a mass basis has been widely accepted, particularly when bivariate relationships are compared with the leaf economic spectrum proposed by Wright *et al.* (Wright *et al.*, 2004). However, there is still controversy (Lloyd *et al.*, 2013, Osnas *et al.*, 2013) about whether the differences observed in the strength of the correlation between mass-based traits and area-based traits (i.e. *A* and N), depends on the fact that both traits are converted using LMA (when expressed on mass basis).

Therefore in this paper we have based our analysis on area based traits. However when considered necessary we have also included comparisons with mass based traits. Photosynthetic and nutrient traits can be converted from a dry weight basis to area basis (or vice versa) using the leaf mass per area. Throughout the text the leaf trait acronym (Table 2) is accompanied by the subscript "mass" to make such distinction, i.e. V_{cmax} refers to the value expressed on an area basis and $V_{\mathrm{cmax\_mass}}$ to the same parameter expressed on a mass basis. Leaf traits included in this study are summarised in Table 2.

Table 2. Abbreviations of the measured traits and their units.

Acronym	**Parameter**	**Units**
V_{cmax}	Maximum rate of carboxylation	µmol CO_2 m^{-2} s^{-1*}
J_{max}	Electron transport capacity	µmol CO_2 m^{-2} s^{-1*}
A_{sat}	Photosynthetic rate at saturating light and ambient CO_2	µmol CO_2 m^{-2} s^{-1*}
g_{S}	Stomatal conductance	mol H_2O m^{-2} s^{-1**}
N_{area}	Leaf nitrogen content	mg N m^{-2***}
C	Leaf carbon content	%
P_{area}	Leaf P content	mg P m^{-2***}
LMA	Leaf mass per area	g m^{-2}
LDMC	Leaf dry matter content	mg g^{-1}
LT	Leaf thickness	mm
N:P	Nitrogen:Phosphorus ratio	

Units on mass basis: * µmol CO_2 g^{-1} $s^{-1,\ **}$ mol H_2O g^{-2} $s^{-1,\ ***}$ mg N or P g^{-1}

Water potential

We measured water potential to assess water limitations to growth. Pre-dawn measurements provide a useful measurement of soil water status (Jones, 1992), and midday measurements are used as indication of the leaf water deficit that species tolerate (Markesteijn *et al.*, 2011).

Pre-dawn (Ψ_{PD}) and midday (Ψ_{MD}) water potential (MPa) were measured for five individuals per species, the same day when the respective *A*/Ci curves were measured. Leaves were collected between 5 and 6 a.m. for Ψ_{PD} and between 1 and 3 p.m. for Ψ_{MD} measurements. Leaves were cut from the top-most part of the tree and sealed into humid plastic bag as described elsewhere (Markesteijn *et al.*, 2011, Brodribb and Feild, 2000), and kept in the dark (a cooler) until measurement. Water potential measurements using this method do not differ significantly from measurements on fresh leaves (Gotsch *et al.*, 2010). Samples were measured within 30 minutes after cutting using a pressure chamber PMS-1000 (PMS Instruments, Oregon, USA). See Figure S2 (Chapter 4b) for Ψ results.

Plant area index

We used plant area index (PLAI) to assess changes in canopy openness across sites and seasons. PLAI (leaf area index + projected are from stems and branches m^2 m^{-2} of ground area) was estimated from hemispherical photos (Nikon D5000 with a Sigma fish eye 4.5mm F2.e EX DC lens). To avoid excess brightness in the images (Jonckheere *et al.*, 2004), photos were taken pre-dawn (before 6 a.m.), at sunset (after 5 p.m.) or under overcast conditions. Photos were taken in a 20 m grid as suggested by Ryan and Williams (2010). Semi-permanent marks were placed so the photos could be taken in the same position during the three seasons. Plant area index was estimated from the digital images using the software Gap Light Analyser 2.0 (Frazer *et al.*, 1999).

Environmental variables

Soil temperature and soil moisture were measured in the morning (between 6 and 8 a.m.) and after mid-day (between 2 and 4 p.m.), on each day that *A*/Ci curves were measured. Soil temperature was measured with a traceable thermometer and soil moisture was measured with a HH2 soil moisture meter (Delta-T, Cambridge, UK). Both measurements were carried out at 5 cm in 5 positions selected randomly within the plot.

Although soil properties at the site have thoroughly been described (Leiva *et al.*, 2009b, Powers *et al.*, 2009a), a compound soil sample was taken from each plot in the rainy season and in the early dry season (see Table S1 in the Chapter 4b), to characterize the site physical and chemical properties.

Half hourly weather data (temperature, relative humidity, wind speed, rainfall and radiation) was provided by the Instituto Meteorológico Nacional of Costa Rica from an automatic weather station located in the Santa Rosa sector (10°50’28’’N, 85°37’10’’ W, 301 m.a.s.l). Vapor pressure deficit (VPD) was estimated from temperature and relative humidity data according to Jones (1992).

Data Analysis

Curve fitting

A/Ci curves were fitted using a maximum likelihood fitting approach in FORTRAN 90 (Intel, Santa Clara, CA, USA), included in the leaf biochemical sub-model of the Soil Plant atmosphere model (Williams *et al.*, 1996), and based on the equations of Farquhar and Von Caemmerer (1982). Curves were fitted with an optimum temperature set to 30^{o}C, which is close to the average measured across seasons.

Fitted values of J_{max} were significantly higher during the TS and the DS (see Figure S4 in Chapter 4b), and the J_{max}:V_{cmax} ratio for the curves during the TS and DS was higher (between 2.76 and 2.96) than values reported as optimum in the literature (between 1.5 and 2.0, (Von Caemmerer, 2000)). As this could be related to the fact that despite high stomatal conductance (Figure 3), photosynthesis did not reach a phase of saturation (Bloomfield, pers. comm.), J_{max} values from TS and DS were excluded from all further analysis.

Statistical analysis

We analysed seasonal-spatial changes in PLAI, water potential and leaf traits (at species level) using a repeated measures analysis of variance (ANOVA) using the software R 2.15.2 (R Core Team 2012). Data were log transformed to meet normality requirements of the test. To test for differences in structural and photosynthesis related traits pooled at plot level we also used a repeated measure analysis of variance but in a mixed effect model using the package lme4 in R (Bates *et al.*, 2013). We used site and field season as fixed factors and a random intercept to address the spatial and temporal correlation between observations (Zuur *et al.*, 2009). Models where compared with a likelihood ratio test for the contribution of a random slope vs. a random slope and intercept (in all cases the simplest model with only the random intercept presented a better fit. Significance of the fixed factors (p values) were estimated using functions in the lmerTest package in R (Kuznetsova *et al.*, 2012).

Comparison of sites within season and seasons across sites was carried out with pairwise *t*-test with Bonferroni adjustment (Quinn and Keough, 2002), or with Tukey adjustment (for PLAI and leaf water potential). Seasonal and spatial changes in the traits were only explored when the seasonal/spatial factor was significant at least at the 5% level ($p<0.05$) in the analysis of variance. Comparison of $J_{\max}$ values was only performed between sites during the rainy season after significant one-way ANOVA.

We explored the relationship between area-based photosynthetic traits and structural traits at leaf level in three ways. First, we used multiple regression to evaluate how relevant N, P and LMA were on the variation of V_{cmax} and $J_{\mathrm{max.}}$ The data were analysed by season and as a combined data set. And we used coefficients of determination and the Akaike Information Criterion (AIC) for model selection. Second, we look at the bivariate relationship of V_{cmax}, $J_{\max}$, A_{sat}, N_{area}, P_{area} using standardized major axis regression (SMA) with the smatr package in R (Warton *et al.*, 2012). Differences on slopes, intercepts and the shift along the axis was compared for all the bi-variate relationships using embedded functions in smatr, that are similar to the comparison of slopes using ANCOVA. Previous to this analysis variables were log-transformed to improve normality (Warton *et al.*, 2006). Finally, we explored the bivariate relationship across the chronosequence, but only for the rainy season when a larger number of repetitions of the photosynthetic traits per site were available. When relevant, comparisons for mass basis data were also explored.

To explore the role of stomatal conductance (g_{S}) and the structural traits on the variation of A_{sat} we used stepwise multiple regression analysis. Additionally, we performed a partial correlation analysis between the predicting parameters to assess the contribution of each factor seasonally and as an entire data set to the observed changes in A_{sat}. A similar analysis was carried out to explore the additional contribution of g_{S} to the variation of the V_{cmax} and J_{max}.

Results

Environmental variables

The measurement periods were characterised by contrasting amounts of rainfall, but air temperature and vapour pressure deficit (VPD) were less variable (Figure S1 Chapter 4b). The rainy season (**RS**, 1st of May to 30th June 2011) was characterised by higher cumulative rainfall (589 mm) than the Transition (**TS**, 10th Nov to 20 December 2011) and the early dry season period (**DS**, 16th January to 10th February 2012) when a minimum amount of rain was recorded (Table 3). An important amount of rain was received during the period between measuring seasons (July-October) when a total of 1700 mm recorded and the largest events were present (Figure S1 Chapter 4b). Average daily temperature remained between 24.3 ºC and 26.1 ºC across the three seasons and it presented a well-defined seasonal pattern (Figure S1 Chapter 4b). Although highest mean temperatures were present during the rainy season, larger values of maximum VPD (Table 3) were recorded during the dry season.

Table 3. Mean temperature; mean maximum vapour pressure deficit (VPD_{max}), mean daily radiation and cumulative rainfall experienced during the 3 measuring periods. Values are the mean and the standard deviation for each period (in parentheses). Data was taken from an automatic weather station located at 10°50’28’’N, 85°37’10’’ W, 301 m.a.s.l. Soil moisture and soil temperature were measured at 5 cm depth on each of the succession sites. Values reported are the average of all sites over the measuring period (n=4).

Field Season	**Temperature (ºC)**	**Rainfall (mm)**	**Radiation (MJ m^{-2} day)**	**VPD_{max} (kPa)**	**Soil moisture (%)**	**Soil temperature (ºC)**
Rainy Season	26.1^{a} (1.3)	589.1^{a}	15^{a} (4)	1.9^{a} (0.6)	33^{b} (12)	26.4^{a} (0.6)
Transition	24.3^{b} (0.8)	66.4^{b}	13^{b} (4)	1.6^{b} (0.5)	31^{b} (10)	23.6^{b} (0.1)
Early Dry season	25.4^{c} (0.9)	5.03^{b}	15^{a} (3)	2.1^{ac} (0.3)	9^{a} (2)	24.2^{b} (0.8)

Values that do not share a letter are significantly different according to pairwise *t*-test with Bonferroni adjustment. Significance set at $p<0.05$.

Soil temperature followed the same pattern as the air temperature and was significantly higher (26.43 ± 0.61 ºC) during the RS. The lowest values were measured during the TS and the DS, although no significant different was observed between the two seasons (Table 3). Soil moisture was 25% higher during the RS and the TS and both periods were significantly different from the DS when soils dried quickly (Table 3).

Plant area index

Plant area index (PLAI) was significantly different between regeneration stages ($F_{29,79}$=29.71, $p<0.001$, Figure 2) and between seasons ($p<0.001$). Maximum values of PLAI were observed during the TS when S_{100} reached a PLAI of 2.41 ± 0.39 [s.d] m^2 m^{-2}. PLAI in S_{65} and in S_{100} were not significantly different until DS (Figure 2) when the S_{100} (mostly dominated by evergreen species) maintained a PLAI 40% higher than $S_{65.}$

S_{15} showed significantly lower values of PLAI across seasons (0.16 to 0.05 m^2 m^{-2}, from the RS to the DS respectively), and it was the only site that did not show a significant decline on PLAI as the dry season approached ($p>0.1$ Tukey Test). However this could be related to the limitations of the methodology to assess PLAI in open areas, as important leaf shedding was observed on the field. S_{25} presented a 70% change from the rainy to the dry season and was significantly different from the late stages of the forest and from S_{15} (Figure 2).

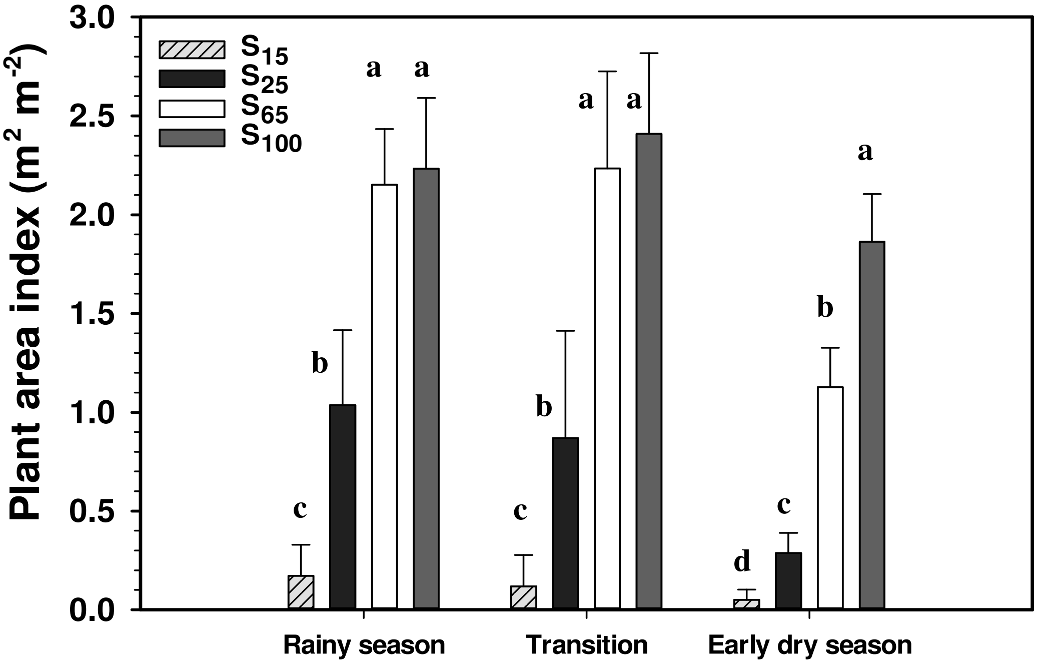

Figure 2. Plant area index (PAI m^2 m^{-2}) for the regeneration sites across the chronosequence. Values are means and standard errors (vertical bars), n=9 for each site. Bars from left to right on each season represent the forest sites (***S***) at the different regeneration ages (15, 25, 65 and >100 years since last disturbance). Within-season PLAI means were compared with a Tukey HSD test at p<0.05 (bars that do not share a letter are significantly different).

Photosynthetic traits

Seasonal patterns of variation

Seasonal variation of maximum rate of carboxylation (V_{cmax}), photosynthetic rate at saturating conditions (A_{sat}), and stomatal conductance at which A_{sat} was estimated (g_S) (Figure 3), was more significant than variations across the chronosequence within season. All pairwise comparisons were considered significant at p<0.05 (Bonferroni-adjusted pairwise t-test [BTT]) and were carried out after significant repeated measurement analysis of variance in a mixed effect model (p<0.05).

When all sites were pooled together across seasons, the analysis of variance showed highly significant seasonal variation (p<0.001) for A_{sat} ($F_{2,95}$=36.90), V_{cmax} ($F_{2,92}$=12.74) and g_s ($F_{2,97}$=30.73), and significant variation (p<0.05) for Ci:Ca

($F_{2,95}$=3.23). However when pairwise comparisons between seasons were carried out across sites, seasonal variation was only significant for A_{sat} and g_s. Not significant differences were observed between the transition and the dry season for any of the photosynthetic traits.

A_{sat} consistently decreased across seasons (Figure 3b), and up to a 60% variation was observed from the rainy season (maximum value of 14.54 ± 1.83 μmol CO_2 m^{-2} s^{-1} measured in S_{25}) to DS. Significant differences in A_{sat} ($p<0.01$, BTT) were observed between the RS and the DS in the S_{15}, and across the 3 seasons in S_{25}. g_S changed significantly on the S_{25} with a 30% decrease from the rainy season to the transition followed by a 49% reduction on the dry season. However, seasonal patterns of variation for A_{sat} and g_S were not significant in the old sites (S_{65} and S_{100}). Maximum average V_{cmax} (80.86 ± 7.41 μmol CO_2 m^{-2} s^{-1}) was present in the transition from the rainy season to the dry season (TS), however it was only 20% than the lowest value reported in RS.

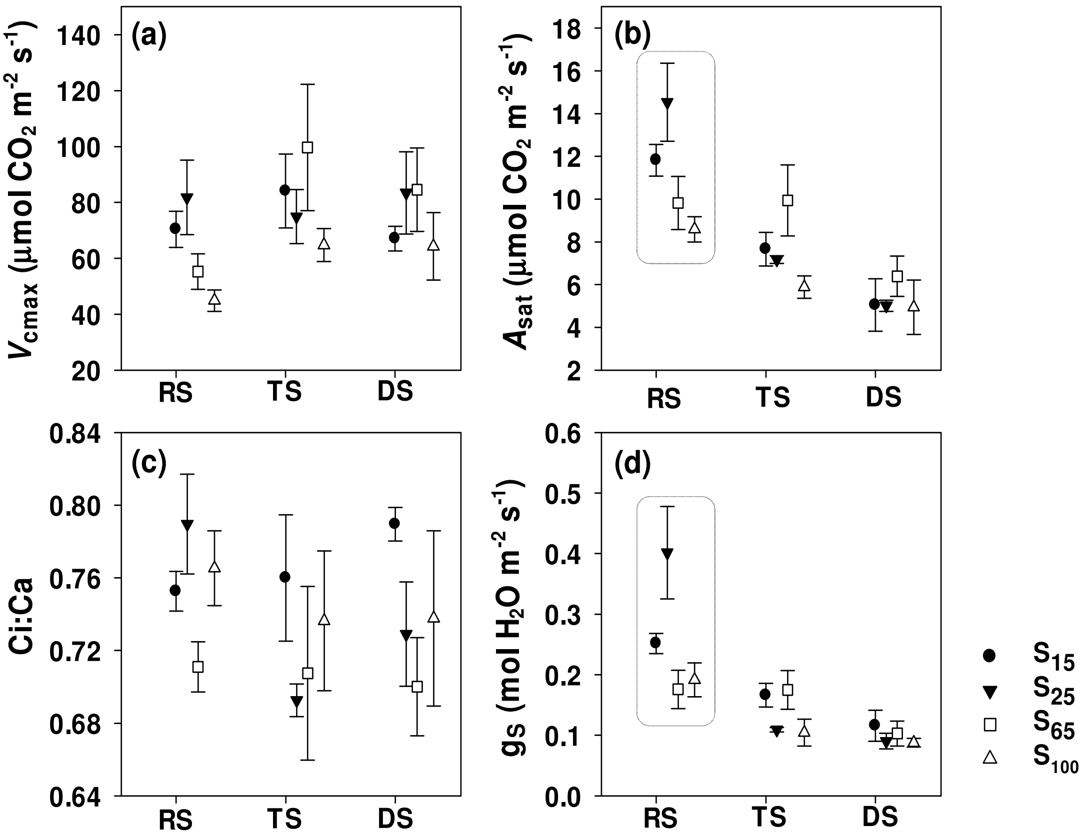

Figure 3. Seasonal patterns of variation of **a)** maximum rate of carboxylation (V_{cmax}), **b)** photosynthetic rate at saturating conditions (A_{sat}), **c)** Intercellular CO$_2$/Ambient CO$_2$ concentration, **d)** stomatal conductance (g_S) at which A_{sat} was estimated. Each value is the average of the species average (n=4 per plot) in the rainy season (RS), the transition from rainy to dry season (TS) and the early dry season (DS).Vertical bars are standard errors. Symbols represent the forest sites (***S***) at the different regeneration ages (15, 25, 65 and >100 years since last disturbance). Seasons when a significant variability was observed across the chronosequence are surrounded with a dotted line.

Variation across the chronosequence

When all sites and seasons were pooled together, the analysis of variance showed significant variation ($p<0.05$) across the chronosequence (significant difference between sites) for A_{sat} ($F_{3,11}=3.44$), and marginally significant variation ($p<0.1$) for g_s ($F_{3,11}=2.63$) and Ci:Ca ($F_{3,11}=3.22$). Variation across the chronosequence for V_{cmax} was not significant, although the interaction between field season and site in the chronosequence was ($F_{6,92}$=2.95). One-way ANOVA showed a significant variation for J_{max} across the chronosequence during the rainy season ($F_{3,102}$=5.67).

Pairwise comparisons for the chronosequence within each season for the photosynthesis-related traits (V_{cmax}, J_{max}, g_s, A_{sat}, Ci:Ca) showed that spatial variation was significant mostly during the rainy season (Figure 3). Most of the significant differences were found between the S_{25} (where the highest values were measured) and S_{100}. No significant difference was found within the young forest stands (S_{15}-S_{25}) or within the old ones (S_{65}-S_{100}) for any of the photosynthesis related traits.

Although V_{cmax} remained fairly constant across the chronosequence within seasons (Figure 3a), and showed no significant difference between sites. During the rainy season V_{cmax} in S_{25} (81.8 ± 13.3 μmol CO_2 m^{-2} s^{-1}) was 40% higher than in S_{100}, site that consistently presented the lowest values of V_{cmax} across seasons.

Changes in J_{max}, A_{sat} and g_S across the chronosequence were significant ($p<0.05$ BTT) during the rainy season, with the highest values found in S_{25}. Maximum J_{max} (Figure S4 Chapter 4b) during the rainy season (148.0 ± 16.1 μmol CO_2 m^{-2} s^{-1}) was measured in S_{25} and it was 48% higher ($p<0.05$ BTT) than the lowest value recorded in S_{100}. J_{max} in S_{15} and S_{25} was also approximately 40% higher than S_{100}. A_{sat} in S_{25} (13.93 ± 1.25 μmol CO_2 m^{-2} s^{-1}) was 40% higher when compared to the S_{100}, and significantly different. Average g_S was 50% higher in S_{25} (0.40 ±0.07 mol H_2O m^{-2} s^{-1}) compared to S_{65}. Ci:Ca did not present significant differences between sites on each season when evaluated.

Although V_{cmax} and J_{max}, were highly correlated during the rainy season (R^2=0.78), a significant shift on their relationship was observed along the axis (Figure 4). This shift translated into a 15% higher J_{max}:V_{cmax} ratio in the S_{15} (2.0 ±0.1), although this difference was only marginally significant ($p<0.1$ BBT).

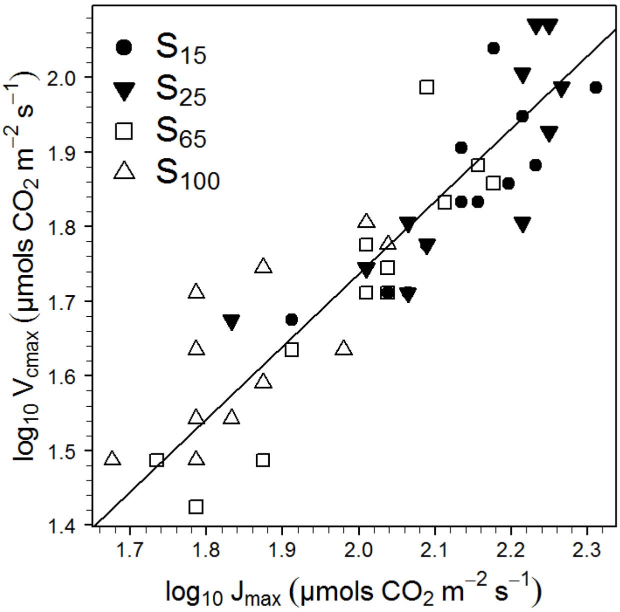

Figure 4. Relationship between maximum rate of carboxylation (V_{cmax}) and electron transport capacity (J_{max}), during the rainy season. The solid line represents the standardized major axis (SMA) regression for $\log_{10}$ transformed data (R^2=0.78). A significant shift along the axis was observed for the young sites (closed symbols) in comparison with the old sites (open symbols). Symbols represent the forest sites (*S*) at the different regeneration ages (15, 25, 65 and >100 years since last disturbance). Pairwise comparisons at p<0.01.

Leaf structural traits

Seasonal patterns

Highly significant season variation (p<0.001) was observed when all sites were pooled together for N_{area} ($F_{2,151}$=26.81), LMA($F_{2,158}$=45.91), N:P ratio ($F_{2,147}$=49,77), N_{mass} ($F_{2,150}$=6,26), and P_{mass} ($F_{2,148}$=48.91). A marginally significant (p<0.1) difference was observed for P_{area}($F_{2,147}$=2.49) . The seasonal patterns of variation on the structural traits (N, LMA and P) seemed to be largely controlled by a trade-off between LMA and nutrient content, where in general a strong negative correlation is observed between LMA, P and N content (Figure S3 a and b, Chapter 4b).

The largest values of LMA were presented in the DS (Figure 5 c) except for S_{100}, which could be associated to the fact leaves of *H.courbaril* were produced just before this measuring season. Pairwise comparisons for LMA showed a significant change (p<0.05 BTT) across seasons for S_{15} and S_{65} , and it is likely that the significant change observed on these sites on LMA is the reason both sites also presented a significant variation on N_{area} across seasons, particularly from the RS to the DS (Figure 5 a). The highest values of N_{area} (1.76 ± 0.23 g m^{-2}) were measured in S_{100} during the transition, but overall this trait presented a low variability across seasons (CV=11%). S_{25} was the only site where a significant change (p<0.05 BTT) on N content (N_{mass}) was observed between the RS and the DS (Figure 5 e). Seasonal variation in P_{area} (Figure 5 b) was not significant on any of the sites. Phosphorus content (P_{mass}) (Figure 5 f) however, presented a decreasing pattern in most of the sites with a general 40% decreased observed from the RS (average 1.0± 0.1 mg m^{-2}) to the DS (0.78± 0.18 mg m^{-2}).

N:P ratio (Figure 5 d) was on average 25 % lower at all sites during the rainy season (average 18.6 ± 0.9), which is more likely associated to the decrease on P_{mass} than to changes on $N_{mass.}$ N_{mass} and P_{mass} were strongly correlated across seasons (R^2=0.63), however an important seasonal variation on this relationship was also observed (Figure S3 c Chapter 4b), which is evident in the changes observed on the N:P ratio.

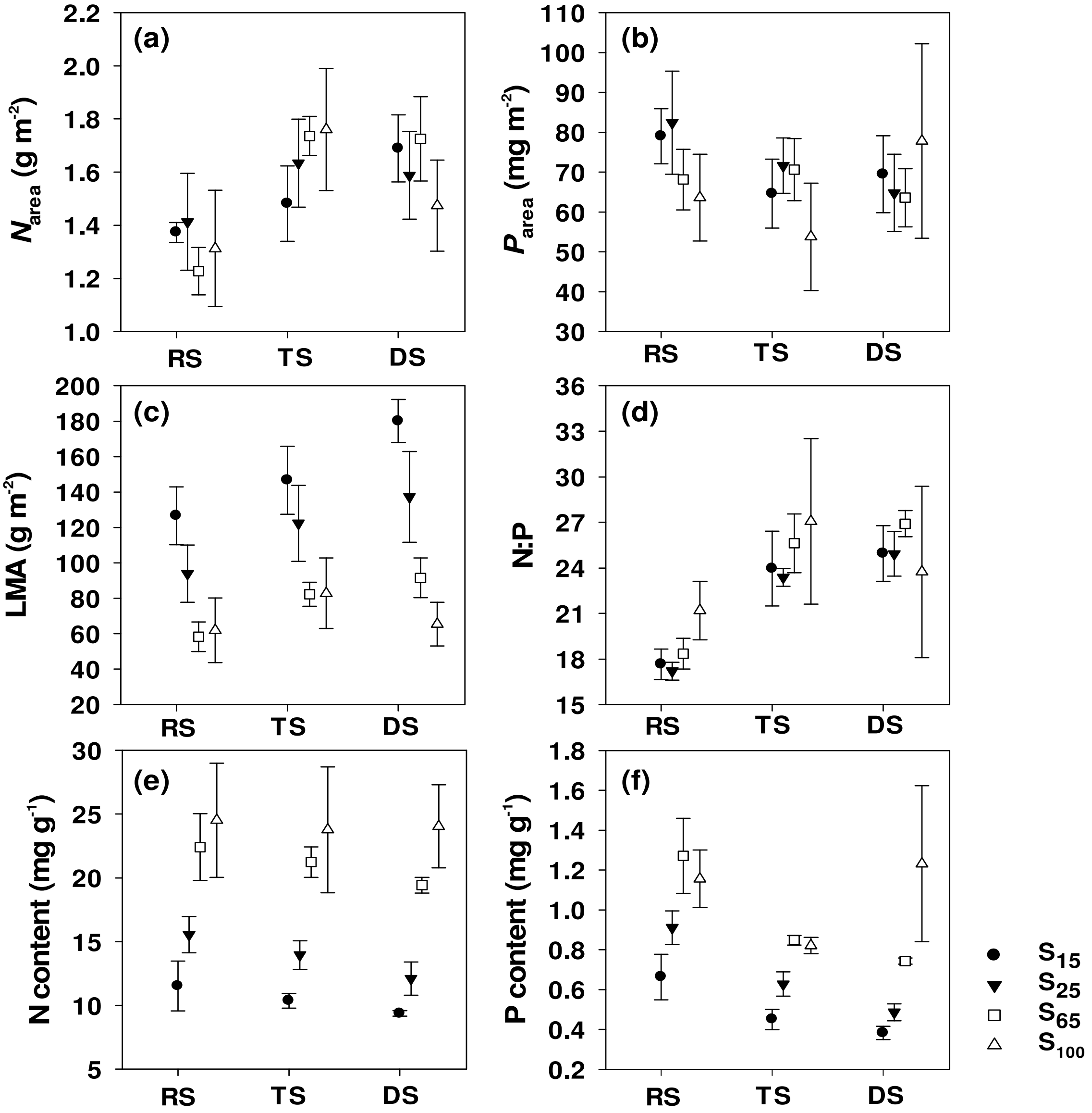

Figure 5. Seasonal patterns of variation of **a)** Nitrogen content on area basis, **b)** Phosphorus content on area basis, **c)** Leaf mass per area (LMA), **d)** N:P ratio, **e)** Nitrogen content on mass basis, **f)** Phosphorus content on mass basis. Each value is the average of the species average at each site (n=4 per plot) in the rainy season (RS), the transition from rainy to dry season (TS) and the early dry season (DS).Vertical bars are standard errors. Symbols represent the forest sites (***S***) at the different regeneration ages (15, 25, 65 and >100 years since last disturbance).

Variation across the chronosequence

When all sites and seasons were pooled together, variation on the structural traits across the chronosequence was highly significant ($p<0.001$), for Nitrogen and Phosphorus on mass basis ($F_{3,12}=3.51$ and $F_{3,12}=14.23$ respectively). Variations across the chronosequence were also significant ($p<0.05$) for LMA ($F_{3,12}=4.84$). However no significant differences between sites were found for N_{area}, P_{area} or N:P ratio.

As with the photosynthetic traits, pairwise comparison of sites within season showed no differences for any of the structural traits within the younger sites (S_{15}- S_{25}) or within the oldest sites (S_{65} -S_{100}). The highest values of LMA were measured in S_{15} across seasons (Figure 5 c), and significant differences ($p<0.05$ BTT), were observed for this site compared to S_{65} and S_{100} in the transition and for S_{100} in the dry season. No significant difference between sites was observed on the rainy season.

Although no significant differences between sites, were observed for N_{area} or P_{area}, spatial variations in N_{mass} and P_{mass} was significant ($p<0.05$for pairwise comparison). N_{mass} was 50% lower in S_{15} across seasons (Figure 5 e) compared to S_{65} and S_{100}, where significant differences ($p<0.05$ BTT) were observed across the 3 measuring seasons. N_{mass} in S_{25} was also significantly lower than the S_{100} during the TS and the DS. P_{mass} was also significantly lower in S_{15} and it was significantly different from the S_{65} and S_{100} (45% lower) during the RS and the TS.

Role of structural traits on the variation of V_{cmax} and J_{max}

V_{cmax} and J_{max} were significantly related to N_{area} and P_{area} in the combined data set but important seasonal variations in the strength of this relationship was observed for V_{cmax} (Table 4). In the rainy season V_{cmax} was more strongly related to P ($R^2=0.45$), but in the TS and the DS was more strongly related to N. No seasonal variations were observed on the slope of the V_{cmax}-N_{area} relationship (Figure 6 a), but a significant shift along the axis was observed (Wald test=23.1, $p<0.001$) for data in the transition and the dry season, what would imply that both traits vary across seasons but their relationship remains the same.

The V_{cmax}-P_{area} relationship (Figure 6 b) was not significant during the dry season, and no significant seasonal differences were observed between the RS and TS in the SMA analysis, but the elevation of the regression was higher for the TS, what would suggest that during the transition leaves have a higher V_{cmax} for a given P_{area} investment. J_{max} was also strongly related to P on the RS (R^2=0.52), but also to N (R^2=0.48) (Figure 6 d and e). Despite the significant control that N and P have on the variation of V_{cmax} and J_{max}, these traits were not significantly related to N:P ratio.

Variations in the relationship of V_{cmax}, J_{max} and A_{sat} with N_{area} and P_{area} along the chronosequence were evaluated only during the rainy season, as a higher number of repetitions within each site were available. The bivariate relationships between V_{cmax}/J_{max} and N_{area}/P_{area} were not significant at the extremes of the chronosequence (S_{15} and S_{100}). For S_{25} and S_{65} no differences in slopes, intercepts or shifts along the axis were observed for the V_{cmax}-N_{area}, V_{cmax}-P_{area} or the J_{max}-N_{area}. However, for the J_{max}-P_{area} relationship, the relative differences in J_{max} observed along the chronosequence (Figure 4) resulted in a significant shift along the axis and species in S_{25} had a higher J_{max} for a given P_{area} in comparison with species in S_{65}. The relationship between A_{sat} and N_{area}/P_{area} was only significant in S_{65} (R^2=0.54 for N_{area} and R^2=0.46 for P_{area}).

The relationship between the photosynthetic traits, leaf mass per area (LMA) and leaf dry weight content (LDMC) were less significant that the observed for N_{area} and P_{area}. For both V_{cmax} and J_{max} the relationship with LMA was only significant in the RS (Figure 6 c and f), when the lowest values of LMA were measured (Figure 5c). Contrary to our expectations, V_{cmax} and J_{max} were not related to changes in LDMC, however, this relationship was significant when the photosynthetic traits were expressed on a mass basis, and it was most significant for V_{cmax_mass} (r=-0.47, $p<0.001$) than for J_{max_mass} (r=-0.20,$p<0.001$) (see Figure S6 Chapter 4b for LDMC results).

Table 4. Coefficients of determination (R^2) for the relationship between V_{cmax}, J_{max} and A_{sat} with leaf nitrogen content (N), leaf phosphorus content (P) and leaf mass per area (LMA). Data is presented for V_{cmax} and A_{sat} across seasons and as combined data set and for J_{max} during the rainy season only. All variables are expressed on an area basis and the data was transformed ($\log_{10}$) previous to the analysis. Coefficients are not shown if they were not significant (N.S., $p<0.05$). See Table S2 in Chapter 4b for regression coefficients, significance values and Akaike Information Criterion.

Model*	V_{cmax}		J_{max}	A_{sat}	
	All data	**Rainy Season**	**Rainy Season**	**All data**	**Rainy Season**
$Y=a+b*N$	0.40	0.39	0.48	N.S.	0.27
$Y=a+b*P$	0.15	0.45	0.52	0.18	0.33
$Y=a+b*LMA$	0.22	0.33	0.51	N.S.	0.21
$Y=a+b*N+c*P$	0.39	0.46	0.56	0.20	0.33
$Y=a+b*N+c*LMA$	0.41	0.42	0.58	N.S.	0.27
$Y=a+b*P+c*LMA$	0.24	0.45	0.58	0.23	0.32
$Y=a+b*N+c*P+d*LMA$	0.40	0.45	0.59	0.22	0.31

*Y represents either V_{cmax} ,J_{max} or A_{sat} n=46 for Rainy season, n=39 for transition, n=28 for Early dry season, n all data=113.

Multiple regressions with N_{area}, P_{area} and LMA explained more variation on the combined data set than the independent regressions, particularly for J_{max} (Table 4). However, the relative contribution of each variable on the prediction of V_{cmax} changed seasonally following the independent traits relationships (see partial correlations coefficients, Table S4 Chapter 4b). The partial contribution of P was more significant in the RS and N was more significant in the late seasons, which explains the pattern observed on N:P ratio (Figure 5 d)

For V_{cmax} the model that included the 3 variables (LMA, N and P) explained 40% of the variance in the entire data set but this was not different from the model including only N. For J_{max}, the model with the 3 structural traits described 59% of the variability on the rainy season, and this was a significant improvement from the models including only one or two structural traits (Table 4). The role of the LMA on these relationships explained why most of the bivariate relationships were highly significant, and the coefficients of determination higher, when variables were expressed on a mass basis (Table S3 Chapter 4b).

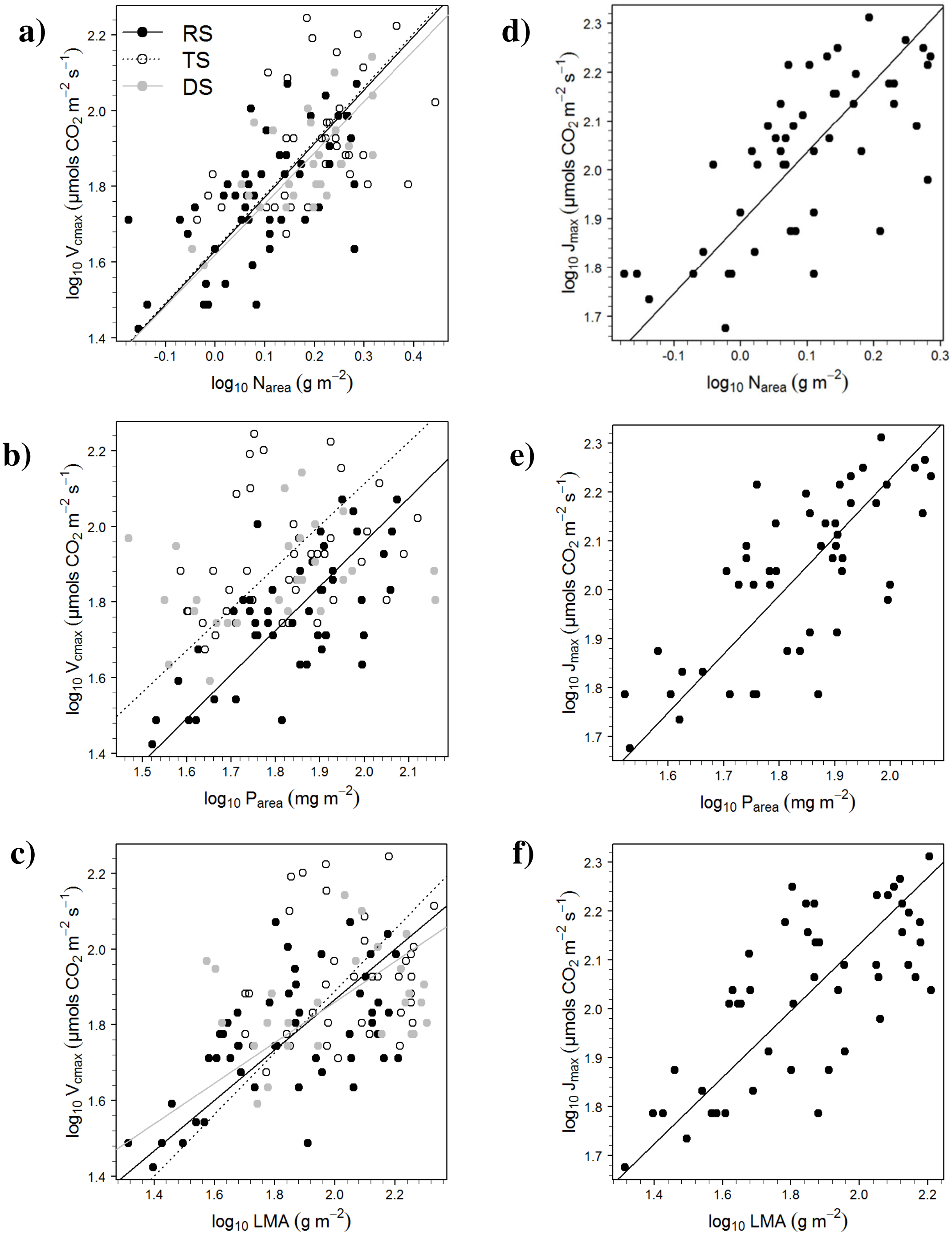

Figure 6. Relationship between maximum carboxylation rate (V_{cmax}), and structural traits during the rainy season (RS), the transition to dry season (TS) and the early dry season (DS). Relationship between electron transport capacity (J_{max}) and structural traits, during the RS. All fits are standardized major axis (SMA) in $\log_{10}$ transformed data expressed on area basis. Regression lines are only plotted if the relationship was significant. **a)** There was not-significant difference between the SMA slopes or the intercepts across season in the V_{cmax}-Nitrogen relationship. **b)** The V_{cmax}-Phosphorus relationship was not significant for the DS, not-significant difference between the SMA slopes were observed between TS and the RS. **c)** The relationship between V_{cmax} and LMA was only significant during the RS. **d)** The J_{max}-Nitrogen relationship, **e)** The J_{max}-Phosphorus relationship and the **f)** The J_{max}-LMA relationship were significant during in the RS. All pairwise comparisons at $p<0.001$. See Table S3 in the Chapter 4b for slopes and coefficients of determination.

Role of stomatal conductance (g_S) and structural traits on the control of A_{sat}

Seasonal variations in A_{sat} followed the observed patterns of variation in g_S (Figure 3 b and d), and both variables were strongly correlated (r=0.70, $p<0.001$) across the entire data set. No significant differences were observed on the Ci:Ca ratio (Intercellular CO_2 /Ambient CO_2 concentration) (Figure 3 c), which further suggests that changes in A_{sat} might primarily be linked to changes in g_S.

To explore the role of N_{area}, P_{area} and LMA on the variation of A_{sat} we also considered g_S as a factor. Independent multiple regressions with N_{area}, P_{area} and LMA independently or combined (Table 4), showed that during the rainy season N_{area} and P_{area} on their own can explain respectively 27% and 33% of the variability, while combined can explain also 33%. However during the early dry season (DS) the role of P_{area} was less significant and N_{area} and LMA combined explained 49% of the variability with a minor increment in AIC from a model including only N. LMA alone did not explain variations in A_{sat}.

When g_S was considered as part of the models, its contribution was significantly larger across seasons and in the entire data set (Table 5), but the contribution of N_{area} and P_{area} was also significant. Along with g_S, P_{area} was the only significant variable during the RS (partial correlation 0.39) and N and LMA were significant during the TS and the DS (Table 5). Our results from the stepwise regression (Table S5 Chapter 4b) showed that all four variables were significant for the entire data set explaining 78% of the variability in $A_{sat.}$

Table 5. Partial correlation analysis for the relationship between A_{sat} and structural traits (N, P, LMA). Data is presented across seasons and as combined data set.

	A_{sat}			
	All data	**Rainy Season**	**Transition**	**Early Dry Season**
g_S	0.81***	0.82***	0.72***	0.75***
N	0.26**	0.21	0.29*	0.72***
P	0.37***	0.39***	0.21	0.09
LMA	-0.36**	-0.12	-0.31*	-0.68***

Correlation for the paired comparison was highly significant***p<0.001, **significant p<0.05 or marginally significant*p<0.1

Although no significant correlation was observed between V_{cmax}/J_{max} and g_S, we also explored the relative contribution of g_S to the variability of these parameters (Table S5 Chapter 4b). The contribution of g_S was only significant for changes in V_{cmax} during the rainy season (partial correlation 0.46) but over the entire data set N_{area} was the only significant parameter. No significant contribution of g_S on the variation of J_{max} was identified during the rainy season.

Intra-specific variation on photosynthetic and structural traits

We explored the variation of photosynthetic (V_{cmax}, J_{max}, A_{sat}) and structural traits (N_{area}, P_{area} and LMA) across the chronosequence (named "intra-specific variability" here onwards), for the four species that were present in at least three sites along the chronosequence. *Redhera trinervis, Semialarium mexicanum,* and *Cochlospermum vitifolium* co-habited S_{15}, S_{25} and $S_{65.}$ *Luehea candida* was also present in S_{25}, S_{65} and S_{100}. Seasonal patterns were highly variable across sites (Figure 7), but in general the patterns were consistent with some of the responses observed at plot level (Figure 3 and Figure 5). A_{sat} and P_{area} experienced a seasonal decrease from the rainy season, N_{area} increased in some species seasonally (probably associated to a significant seasonal change in LMA in most species), and V_{cmax} remained fairly constant with the exception of *S. mexicanum* (Figure 7). Therefore, in this section we only discuss the intra-specific variability (seasonal patterns are summarised in Table S6 in the Chapter 4b). All pairwise comparisons were considered significant at $p<0.05$ (Bonferroni-adjusted pairwise t-test [BTT]) and were carried out after significant repeated measures ANOVA (or one-way ANOVA for J_{max}), when the contribution of the variability across the chronosequence was significant ($p<0.01$).

Values of A_{sat} were highly constrained across sites within seasons, and intra-specific variability was only significant for *L. candida* ($p<0.05$ BTT). This species, presented a 50% difference of the lowest values measured in S_{100} and highest value (6.53± 0.38 μmol CO_2 m^{-2} s^{-1}) measured in S_{65} during the dry season (DS) (Figure 7).

V_{cmax} was more variable than A_{sat} across the chronosequence, and in most cases the highest values were measured in $S_{65,}$ either during the transition from the rainy season to the dry season (TS) or at DS (Figure 7). However, significant intra-specific variability was only observed for *L. candida* that when it was 65% higher in S_{65} (55.57± 1.19 µmol CO_2 m^{-2} s^{-1}) in the DS compared to S_{100}.

Intra-specific variability in N_{area} was only significant during the TS for *L. candida* and *C. vitifolium* and in both cases the highest values were measured in S_{65} (Figure 7). N_{area} in *L. candida* in S_{65} (1.56 ±0.09 g m^{-2}) was 30% higher than the values measured in S_{100}. A similar difference was observed for *C. vitifolium,* as N_{area} estimated in S_{65} (1.69 ± 0.161 g m^{-2}) was 36% higher than in S_{15} and 20% higher than S_{25}.

P_{area} presented significant intra-specific variability for *S. mexicanum* and *R. trinervis.* For *S. mexicanum*, average values of P_{area} during the RS in S_{25} (85 ± 7 mg m^{-2}) were significantly higher (20%) than in S_{65}. For *R. trinervis* (Figure 7), P_{area} was 42% higher in S_{25} (compared to 66 ± 8 mg m^{-2} measured in S_{65}) during the RS, and during the TS P_{area} was 29% higher in S_{15}, compared to S_{65} (63 ± 7 mg m^{-2}).

LMA change significantly across the chronosequence every field season (Figure 7), except for the *C. vitifolium* during TS. However, no significant differences were observed within the young stands (S_{15}-S_{25}) or the old stands (S_{65}-S_{100}) for any of the species. Values measured for *R. trinervis* and *S. mexicanum* in S_{15} and were on average 47% higher than in S_{65}, whilst LMA in S_{25} were 46% higher than in S_{65} for *R. trinervis* and 28% higher for *S. mexicanum.* For both *L. candida* and *C. vitifolium* (only during RS) the difference observed between the young and the old stands was approximately 30%.

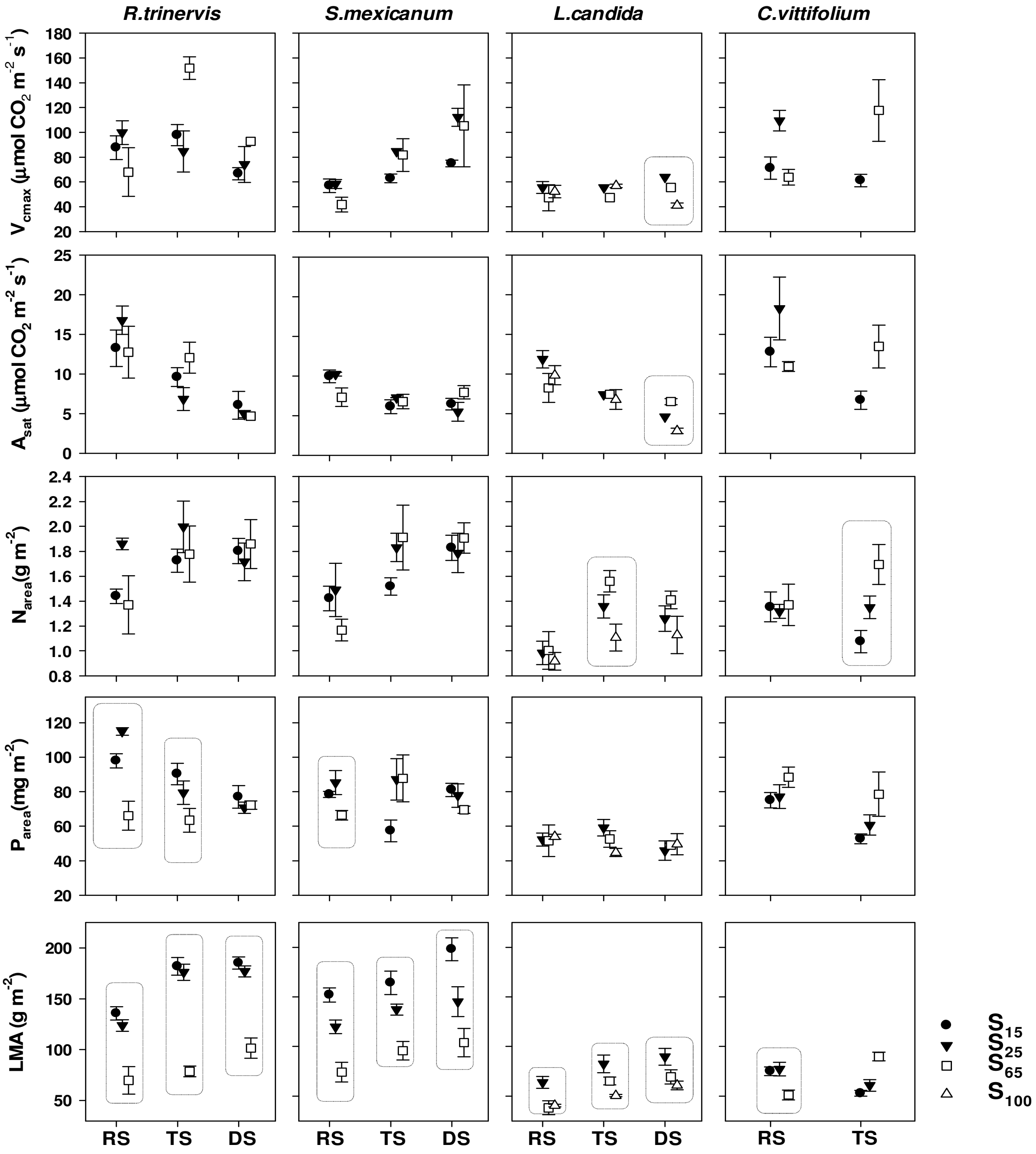

Figure 7. Intra-specific variation on maximum carboxylation rate (V_{cmax}), assimilation rate at saturating conditions (A_{sat}), Nitrogen (N_{area}) and Phosphorus content (P_{area}) on area basis area for *Redhera trinervis, Cochlospermum vitifolium, Semialarium mexicanum* and *Luehea candida.* Data represent averages and standard error (vertical bar) for at least n=3 across the rainy season (RS), the transition (TS) and the early dry season (DS), except for *C. vitifolium* that had shed all its leaves by the DS. No error estimates are shown for photosynthetic traits of *L. candida* in S_{25} and S_{65} during RS and TS, as only two measurements were available. Symbols represent the different succession stages (S) in the chronosequence (15, 25, 65 and >100 years after disturbance). Seasons when significant intra-specific variability was observed area surrounded with a dotted line.

Although the trend in our $J_{\max}$ results (Figure 8) suggest that during the rainy season *C. vitifolium, R. trinervis* and *S. mexicanum* present higher values of $J_{\max}$ in more open areas (i.e. S_{15} and S_{25} compared to S_{65}), no statistically significant intra-specific variability on $J_{\max}$ was observed for any of the species evaluated.

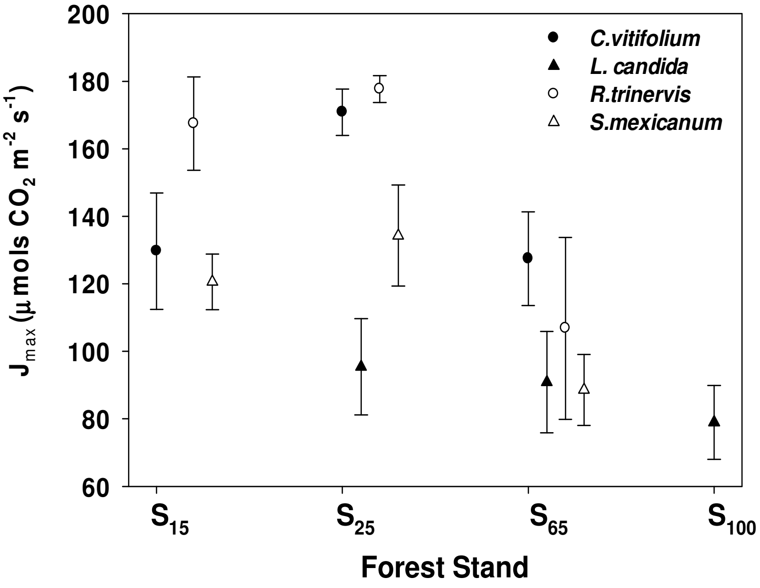

Figure 8. Intra-specific variation on electron transport capacity ($J_{\max}$), for *Redhera trinervis*, *Cochlospermum vitifolium*, *Semialarium mexicanum* and *Luehea candida* along the chronosequence during the Rainy Season. Data represent averages and standard error (vertical bar) for at least n=3. Symbols represent species. Values on the x axis represent the different forest stands (*S*) in the chronosequence in age order (15, 25, 65 and >100 years after disturbance).

Discussion

N and P covary in the seasonal control of photosynthetic parameters

Our results support the hypothesis that V_{cmax} is strongly linked to variations in N_{area} (H1.1). Seasonal variation in N_{area} was more significant than for V_{cmax} and although the V_{cmax}-N_{area} relationship was stronger during the rainy season (Table 4), the significant change along the axis of this relationship (Figure 6a), further confirms that both traits co-vary across the seasons but their relationship remains the same. V_{cmax} was also strongly related to P during the rainy season (Table 4), however the significant difference in the V_{cmax}-P_{area} relationship across seasons suggest that species are able to deploy the same V_{cmax} with a relatively lower P (Figure 5 b). Contrary to commonly predicted ecophysiological relationships, during the rainy season, J_{max} was equally controlled by both N_{area} and P_{area} (Figure 6 and Table 4), what contradicts our hypothesis (H1.1).

The values reported here for V_{cmax} (26.57- 175.71 µmols CO_2 m^{-2} s^{-1}) and J_{max} (47.43-205.14 µmols CO_2 m^{-2} s^{-1}), are consistent with the scarce reports from other tropical sites (Meir *et al.*, 2007, Domingues *et al.*, 2012, van de Weg *et al.*, 2012, Woollen, 2013) with a slope for the V_{cmax} :J_{max} relationship of 0.97 and 1.17 when expressed on area or mass basis respectively (Table S3 supplementary material). In addition, although the observed relationships between $V_{\mathrm{cmax}}/J_{\mathrm{max}}$ and $P_{\mathrm{area}}/N_{\mathrm{area}}$ falsify our main hypothesis (H1.1), these results coincide with findings from other tropical sites that suggest neither N or P limit photosynthetic metabolism, but that it could be a combination of both factors (Domingues *et al.*, 2010, Fyllas *et al.*, 2009, Meir *et al.*, 2007).

It has been suggested that under conditions of relatively low P supply and high N supply, plants take up more N than P what translates in high N:P ratios (Koerselman and Meuleman, 1996). Where N:P ratios above 16 (Reich and Oleksyn, 2004), as the observed in our site, (Figure 4d), usually reflect that species are strongly P limited. The existence of a P limitation on our study site, long suggested for tropical forests (Vitousek, 1984, Vitousek *et al.*, 2010), is further supported by the low soil P found across seasons along the chronosequence (Table S1 Chapter 4b). However, it is likely that this factor only enhances species efficiency for the resorption of P (Wright and Westoby, 2003, McGroddy *et al.*, 2004), and that seasonal differences observed on the covariation of the control of P and N over V_{cmax} and J_{max} area a direct result of this.

Additionally, it is likely that the observed variations in the N:P ratios reflect internal nutrient translocation, particularly of P. Translocation is likely considering that mature leaves are still photosynthetically active but no longer growing, which greatly reduces the P requirements for RNA, so that P can be mobilized to other sinks (Gusewell, 2004). This would also explain why the strong contribution of P is only observed in the RS, or when the leaves have been recently produced (i.e. *H. courbaril* during the DS, Table S8 b Chapter 4b). However, the decrease observed in P during the transition, for the other species, could also be a response to the need to allocate N and P to potentially activated drought metabolic pathways (Domingues *et al.*, 2010) that would be needed to cope with the reduced precipitation during July-August (Figure S1 Chapter 4b) and the rapid soil drying once the rain stops (O.J. Rocha, unpublished data).

Stomatal conductance, N and P control seasonal variation on A_{sat} but not on V_{cmax}

Our results showed that seasonal variation in A_{sat} is strongly controlled by changes in g_{S} (compare Figure 3 b and a). The significant seasonal variation observed in g_{S} is likely associated to the significant decrease in rainfall (Table 3) and to soil drying (evidenced in the higher Ψ_{PD} values during the early dry season), but not to changes in VPD (as we suggested in our alternative hypothesis H1.2), as it seems that this factor is likely to change much later in the year (probably between February and April, see Figure S1 Chapter 4b), when the leaf shedding for most of the deciduous species is expected (Brodribb and Holbrook, 2007).

Although N_{area} and P_{area} also explained an important amount of the observed variability in A_{sat} (particularly during the rainy season), their contribution was not as significant as the g_{S} (Table 5), what contradicts our hypothesis (H1.1). The clearly seasonal pattern observed in A_{sat}-N_{area} and A_{sat}-P_{area} would probably explain why when we compared the slope of these two bivariate regressions with the slopes reported in the global data set by Wright *et al.* (2004), we found that the A_{sat}-N_{area}

slope of the RS data set (Table S3 Chapter 4b) was not significantly different from the one reported by Wright but the A_{sat}-P_{area} was. Furthermore, in the dry season the opposite pattern occurs. Overall, of the models used to explore the variation on A_{sat}, the one that included both nutrients and LMA explained up to 73% of the variability in the A_{sat} (Table 4).

The limited seasonal variation observed within sites on V_{cmax}, despite important variations on g_s, (Figure 3 a and d), is likely associated to the fact that the decrease in Rubisco activity has been reported to occur at values of g_S relatively lower (<100 mmol H_2O m^{-2} s^{-1}) (Flexas and Medrano, 2002) than the g_S reported here. Our results however coincide with findings by Domingues *et al.* (2012), who reported no significant differences in V_{cmax} from the rainy to the dry season of species in an eastern Amazonian rain forest.

Variation in the photosynthetic-nutrient relationships along the chronosequence

Our exploration of the bivariate relationships along the chronosequence during the rainy season showed that the relationship between V_{cmax} and N_{area}/P_{area} and between J_{max}-P_{area} is not significant at the end of the chronosequence (S_{15} and S_{65}). This finding supports our alternative hypothesis (H1.2), and is consistent with findings by Meir *et al.* (2007), along different successional stages in the Sahel.

It is not clear why the photosynthetic-nutrient relationships decoupled on these sites; however a few explanations could be suggested. First, the evergreen species measured in the S_{100} (*H. courbaril*) had its major leaf flushing event during the transition from the rainy season to the dry season (per. ob.), whilst the other species included in this plot (*L. candida* and *E. mexicanum*) are deciduous species whose main leaf flushing event happens with the onset of the rains. These timings of leaf flush would mean that although no major seasonal differences were observed in V_{cmax} at plot level (Figure 3) the existence of different contents of N and P (P is significantly higher in the DS, Figure 5 d) at species level could decouple this relationship. Second, *H. courbaril* (largely dominant in S_{100}, Table 1) is the only N-fixing species within the group studied which means that a more steady supply of N could be available affecting the N/P relationship not only of this species but other species in the same area (Gei and Powers, 2013, Powers and Tiffin, 2010). Third, the decoupling of the relationship in S_{15}, could be caused by N being allocated not only to photosynthesis but also to other tissues for protection against herbivory and desiccation (Vitousek and Sanford, 1986), particularly *C.vitifolium* that flushes its leaves before the onset of the rains (Fallas-Cedeno *et al.*, 2010). However, the fact that the decoupling of this relationships could be caused by intra-specific variability or the combination of species displaying different sets of traits, should not be excluded.

The pattern of variation observed for the J_{max}-P_{area} relationship, where species in S_{25} had a higher J_{max} for a given P_{area} in comparison with species in S_{65} (also supporting our alternative hypothesis H1.2), further support the idea that species on sites with limited P availability are likely to be more efficient in the use of P as was previously suggested for the seasonal control. However, the relative differences (not significant) observed for the different species in their J_{max} along the chronosequence (Figure 8) could also be driving this pattern.

Limited intra-specific variability of photosynthetic leaf traits

The patterns of intra-specific variation (changes in leaf traits along the chronosequence) for *R. trinervis*, *C. vitifolium*, *S. mexicanum* and *L. candida*, followed the patterns observed at plot level, and in general variation in structural traits was larger than that observed in the photosynthetic parameters, which supports our hypothesis (H2.1). We expected that the pioneer species would benefit from less competition for resources in S_{15} and S_{25} (less light competition in particular due to a lower PLAI, Figure 2). However, there was no evidence of higher values in their photosynthetic traits ($V_{cmax,}$ J_{max} and A_{sat}) (Figure 7 and Figure 8), which falsifies the second statement in our hypothesis (H2.1).

Our results suggest that species along the chronosequence share the same controlling factors on their photosynthetic traits, particularly the role of g_S on A_{sat} (Brodribb and Holbrook, 2003, Gutierrez-Soto *et al.*, 2008) despite changes in N and P availability. The significance of our analysis might be limited by the small number of within species repetitions (n=3), however. The existence of a common response is further supported by the fact that despite differences in water availability across the succession (more available water in S_{15} and S_{25}, Leiva *et al.* (2009b)) and expected differences in rooting depth, the species share a similar response to changes in water potential (Figure S2 in Chapter 4b).

However, the mild differences observed in A_{sat}, V_{cmax} and J_{max} for *R. trinervis* and *C. vitifolium* (Figure 7 and Figure 8) during the transition, could also be related to leaf age. Leaves in the S_{65} (where the values were higher) might have been produced a couple of weeks later than in S_{15}/S_{25}. And relative differences in leaf age might be important for parameters like A_{sat} and N content, as important variations can be observed within a matter of weeks as the leaf ages (Gotsch *et al.*, 2010, Chinchilla-Soto unpublished data).

Intra-specific variability on structural traits and implications for nutrient economics

Contrary to the limited variability observed on the photosynthetic traits, individual species showed a significant intraspecific variability in their structural traits, particularly in the P_{area} and the LMA, and both seasonal (Table S7 Chapter 4b) and spatial variation (Figure 7) were significant. Although the number of within species repetitions (n=5 in most cases) is lower than recommended (de Bello *et al.*, 2011), our data coincides with previous leaf traits analysis on this forest that suggest that both forest type and season are important to explain variation in LMA, N and P content, but also the intra/inter specific variability in structural traits is high (Gotsch *et al.*, 2010, Powers and Tiffin, 2010).

The plot level data (Figure 5) clearly reflects the combination of species with different leaf traits (what supports our hypothesis H2.1). For example, the LMA in S_{15} (Figure 5 c) is higher than in S_{25}, although at species level the two sites look similar (Figure 8). However when the species data is explored, it is clear that the difference observed at plot level are caused by the species that is different among the two sites (i.e. during RS average LMA for *L. candida* in S_{25} is 63.75 ± 6.11 g m^{-2} and for *B. crassifolia* in S_{15} is 139±0.4 g m^{-2}). The seasonal variability observed in LMA could also be related to the plasticity of this trait (Poorter *et al.*, 2009), or to lignin accumulation as leaf ages (Brodribb and Holbrook, 2003), which has been suggested as one of the reasons for the decrease in hydraulic conductivity on SDTF species.

The patterns observed for the nutrients are less clear than the LMA trends and species that presented significant intra-specific variability in N_{area} did not present significant variability in P_{area}. However, it is likely that the observed differences in P_{area} along the chronosequence in *R. trinervis* and *S. mexicanum* are the combination of the relative differences observed in the P content (Table S8 Chapter 4b) and the presence of more sclerophyllous leaves in S_{15} and S_{25}. Although at plot level P_{area} did not change significantly across seasons, at species level changes in P_{area} were significant for the three pioneer species (*R. trinervis, S. mexicanum* and *C. vitifolium*), which would suggest the existence of an efficient P remobilization, and that P content at leaf level is related to growing conditions (Hedin *et al.*, 2009, Fyllas *et al.*, 2009).

Our results for N certainly confirm the suggestion that stress and disturbance select for nutrient-restricted sites species with low specific leaf area (high LMA), low leaf nitrogen content but high leaf dry matter content (LDMC)(Sonnier *et al.*, 2010). The concentration of N in these leaves (0.9-2%) are in the range of values previously reported for this site (Powers and Tiffin, 2010) and SDTF in other regions (Jaramillo and Sanford, 1995, Lal *et al.*, 2001). Although the remobilization of N is not as evident as with P, it is a well know phenomena that could happen later in the year (before leaf shedding) and that could be up to 73% in the SDTF (Lal *et al.*, 2001), with major implications for nutrient cycling on the ecosystem. Particularly considering that C:N ratios found in in S_{15} (44.1 ± 1.4) and S_{25} (34.2 ± 1.2), were almost double those in S_{65}-S_{100}, what suggests the material in younger sites is likely to be harder to decompose (especially if microorganisms are N-limited). Additionally, as processes of decomposition/mineralization in the SDTF are more water than temperature limited (Powers *et al.*, 2009b), it is likely that deciduous species, in particular, need to be more efficient in the remobilization processes, particularly temporary storage for future growth (Hikosaka, 2005, Kikuzawa and Lechowicz, 2011).

Conclusions

Overall our results show that V_{cmax} and J_{max} are strongly linked to variations in N and P. However, the strength of these relationships is affected by seasonal nutrient variation and by plasticity in species response to the different stages of the succession (particularly changes in leaf mass per area). The limited variation observed between the younger sites (S_{15}-S_{25}) and between the older sites (S_{65}-S_{100}) suggests that the species selected in those guilds share a similar group of traits (particularly photosynthetic traits), which could simplify the sampling strategy for up-scaling purposes.

The seasonal differences we found make clear the importance of observing seasonal trends in these forests. Data from the transition or the early dry season leaves display a completely different set of trait relations (i.e. low A_{sat}, same V_{cmax}, high N_{area} and LMA, and low P_{area}) that would result in different conclusions if the data were used to predict productivity over a longer period of time. Additionally our results highlight the care that is needed when generalized assumptions of other ecosystems (i.e. V_{cmax}-N relationship) are extrapolated to the dry tropics, where leaf age, key to determining allocation processes, should be considered (Shipley *et al.*, 2005).

Our results made clear that despite the favourable hydrological conditions provided for the gas exchange measurements, stomatal control was the main driver of variation in A_{sat} across seasons, overriding the role of N. This control was particularly important in the transition and the dry season, when the co-ordination of hydraulics and photosynthesis is expected to be stronger through a more optimal stomatal regulation (Brodribb *et al.*, 2002).

Our intra-specific data analysis (along the chronosequence) was certainly limited by the difficulty in obtaining a relatively high number of successful V_{cmax}, J_{max} and A_{sat} values. However, we provide here the first report of these parameters for these ecosystems, and how they can vary across successional gradients. On the other hand, our structural traits are consistent with results by Markesteijn *et al.*(2007), Powers and Tiffin (2010) and Hulshof and Swenson (2010) among others, that report high trait diversity within and between species in the SDTF.

The limited variation observed on the photosynthetic traits, the high diversity found in the structural traits, and the uneven seasonal variation of both sets of traits, is perhaps one of the main reasons why generalizations in the bivariate relationships between photosynthetic parameters and structural traits might not hold as predicted (Wright *et al.*, 2004) in the SDTF (Domingues *et al.*, 2012, Gotsch *et al.*, 2010). Challenges to simple relationships arise particularly because of the existence of species with “unusual” traits combination (Reich *et al.*, 2007), that in the SDTF includes the existence of evergreen species with short life span (less than a year), the large abundance of N fixing species that modify their own internal regulation and modify available resources for species nearby (Gei and Powers, 2013), and the existence of species that need to allocate their nutrients (N in particular) not only to photosynthesis but also to structural protection.

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Chapter 4b. Spatial and seasonal variability of photosynthetic parameters and leaf traits in a Neotropical seasonally dry forest - Supplementary material

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Environmental Variables

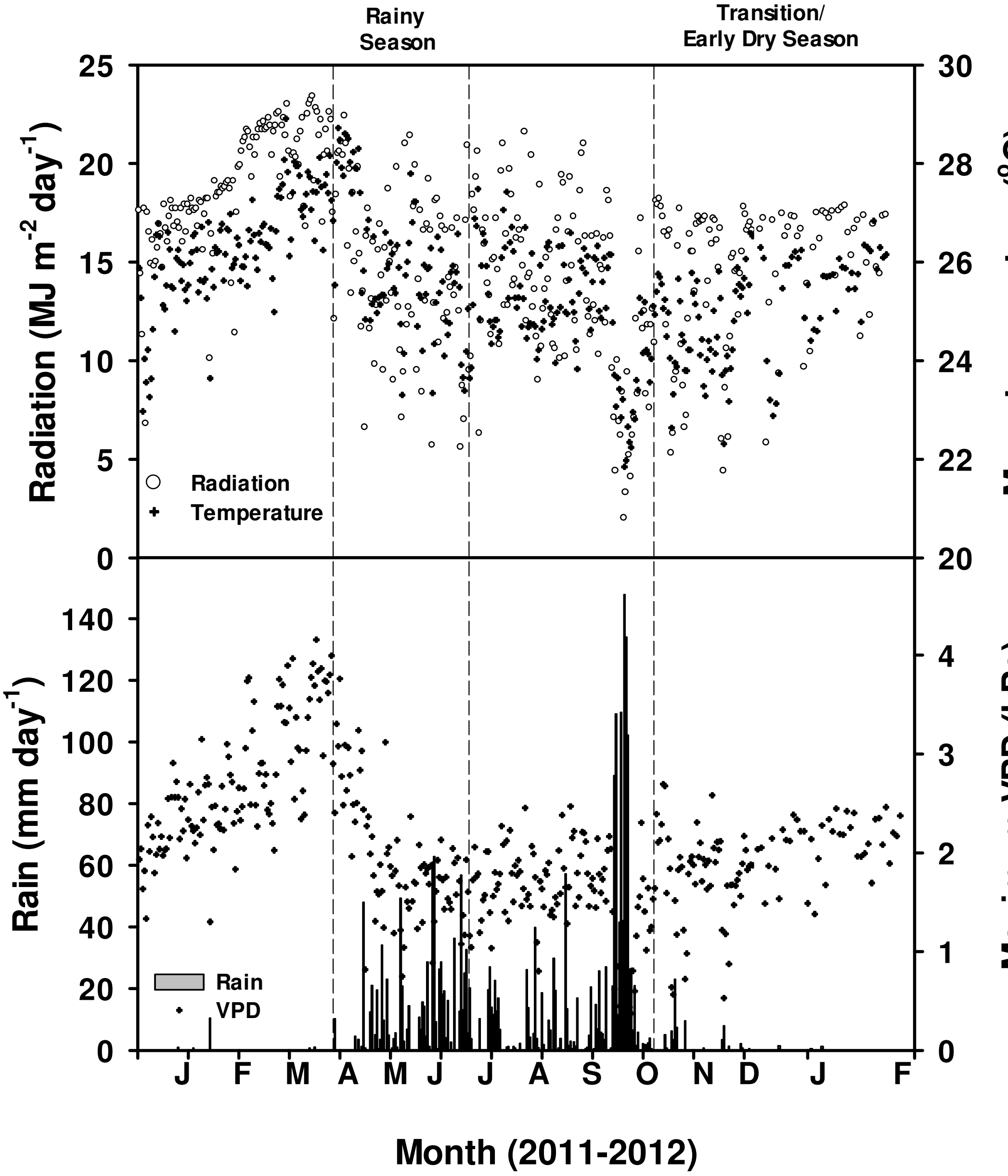

Figure S 1. Mean daily radiation, mean maximum temperature, daily rainfall and mean maximum vapour pressure deficit (VPD) experienced during the 3 measuring seasons. Vertical dotted lines separate the measuring seasons: Early rainy season (1st of May to 30th June 2011), Transition from rainy to dry season (10th November to 20th December 2011) and early dry season (16th January to 10th February 2012). Data was taken from an automatic weather station located at 10º 50'28" N, 85 º 37'10" W, 301 m.a.s.l.

Table S 1. Soil phosphorus content, nitrogen content and soil organic matter (SOM) and soil texture along the chronosequence. Chemical properties were measured in the rainy season (RS) and the early dry season (DS). Texture was only measured in the RS.

Site	P (mg l^{-1})		N (%)		SOM (%)		Texture (%)			Dominant textural class
	RS	DS	RS	DS	RS	DS	sand	silt	clay	
S_{15}	ND	ND	0.2	0.25	3.7	4.3	50	30	20	Loam
S_{25}	1	ND	0.2	0.17	4.2	3.2	34	33	32	Clay loam
S_{65}	1	1	0.4	0.48	6.8	5.2	55	30	15	Sandy loam
S_{100}	2	1.5	0.34	0.38	4.7	7.5	60	28	12	Loam

Water potential

Predawn water potential (Ψ_{PD}) (Figure S 2 a), changed significantly across seasons ($p<0.001$), and a significant interaction between season and regeneration site was found ($p<0.02$). Differences between regeneration sites, although significant, were not so large until the dry season (DS) when the highest value -2.48 ±0.21 MPa was measured in S_{25}, and it was 60% higher than the values measured in S_{15} and 48% higher than values in S_{65} and S_{100}. Values measured in DS were up to 5 times higher that values reported in the previous seasons.

Midday water potential (Ψ_{MD}) (Figure S 2 b) presented a similar pattern as $\Psi_{PD.}$ There were significant differences between seasons ($p<0.001$), however, the interaction between seasons and sites was marginal ($p<0.1$). For differences evaluated on each season, during the rainy season Ψ_{MD} in the S_{15} was in average 20 % higher than for the other sites. During the transitions and DS all regeneration sites were on average 35% higher than S_{100} (Figure S 2B). The largest values of Ψ_{MD} where observed for all the sites on the dry season when as with the predawn measurements the maximum value (-2.81 ± 0.28 MPa) was reached at the S_{25}. On average, Ψ_{MD} values were between 35% (S_{100}) and 72% (S_{15}) higher than the correspondent Ψ_{PD} values.

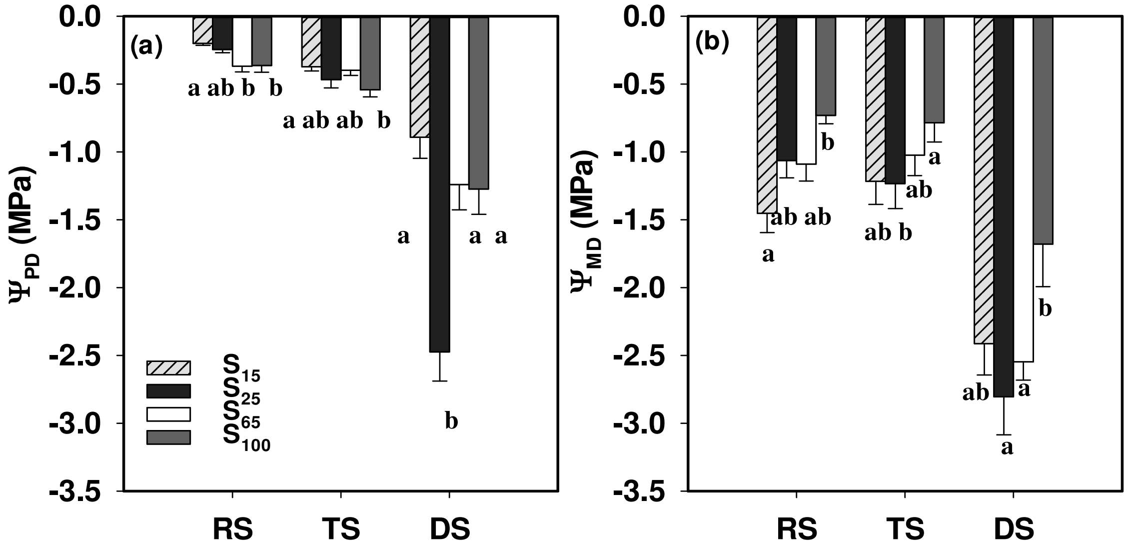

Figure S 2. **a**) Predawn (Ψ_{PD}) and **b**) midday (Ψ_{MD}) water potential for the four regeneration sites during the rainy season (RS), the transition into dry season (TS) and the early dry season (DS). Values are means and standard errors (vertical bars). Bars from left to right on each season represent the forest sites (***S***) at the different regeneration ages (15, 25, 65 and >100 years since last disturbance).Within-season Ψ_{PD} and Ψ_{MD} means were compared with a Tukey HSD test at $p<0.05$. Bars that do not share a letter are significantly different.

Role of structural traits on the variation of V_{cmax}, $J_{\max}$ and A_{sat}

Table S 2. Regression coefficients, coefficients of determination (R^2) and Akaike Information criterion (AIC) for the relationship between V_{cmax}, $J_{\max}$ and leaf traits (nitrogen content [N], phosphorus content [P] and leaf mass per area [LMA]. Data is presented for V_{cmax} across seasons and as combined data set and for $J_{\max}$ during the rainy season. All variables are expressed on an area basis and the data was $\log_{10}$ transformed previous to the analysis. Coefficients are not shown if they were not significant (N.S. $p<0.05$).

	V_{cmax}				$J_{\max}$
Model*	**All data**	**Rainy Season**	**Transition Rainy - Dry Season**	**Early Dry Season**	**Rainy Season**
$Y=a+b^{*}$ N	*a*=-0.98, *b*=0.90 $R^{2=}$0.40, AIC=-138.5	*a*=-1.01, *b*=0.89 $R^{2=}$0.39, AIC= -57.3	*a*=-0.11, *b*=0.63 $R^{2=}$0.17, AIC= -37.9	*a*=-0.96, *b*=0.88 $R^{2=}$0.40, AIC= -41.6	*a*=-1.14, *b*=1.02 $R^{2=}$0.48, AIC=-63.00
$Y=a+b^{*}$ P	*a*=1.03, *b*=0.44 $R^{2=}$0.15 AIC=-99.84	*a*=0.31, *b*=0.79 $R^{2=}$0.45 AIC=-62.1	*a*=1.28, *b*=0.35 $R^{2=}$0.08 AIC=-33.44	N.S.	*a*=0.42, *b*=0.87 $R^{2=}$0.52 AIC= -66.99
$Y=a+b^{*}$ LMA	*a*=1.19, *b*=0.33 $R^{2=}$0.22,AIC=-109.44	*a*=1.04, *b*=0.39 $R^{2=}$0.33,AIC=-52.97	N.S.	N.S.	*a*=1.12, *b*=0.49 $R^{2=}$0.51,AIC= -65.63
$Y=a+b^{*}$ N$+c^{*}$ P	*a*=-0.97, *b*=0.88, ***c***=0.01 R^2=0.39,AIC=-136.53	*a*=-0.40**,** *b*=0.38, ***c*=0.54** R^2=0.46,AIC=-62.53	*a*=-0.05,***b*=0.56**, *c*=0.08 R^2=0.15,AIC=-36.07	*a*=-1.53, ***b*=1.21**, *c*=-0.25 R^2=0.45,AIC=- 2.33	*a*=-0.51, *b*=0.49 *c*=**0.56** $R^{2=}$0.56,AIC=-69.72
$Y=a+b^{*}$ N$+c^{*}$ LMA	*a*=-0.80, ***b*=0.78**,*c*=0.08 R^2=0.41,AIC=-38.14	*a*=-0.49, ***b*=0.62**,*c*=0.19 R^2=0.42,AIC=-8.51	*a*=-0.11, ***b*=0.62**,*c*=0.03 R^2=0.16,AIC=-5.96	*a*=-1.32, ***b*=1.08**, *c*=-0.12 R^2=0.42,AIC=-42	*a*=-0.28, *b*=0.56,*c*=0.31 R^2=0.58 AIC=-71.81
$Y=a+b^{*}$ P$+c^{*}$ LMA	*a*=0.96, =0.21,***c*=0.26** R^2=0.24, AIC=-111.01	*a*=0.38, ***b*=0.65,** *c*=0.10, R^2=0.45,AIC=-0.92	N.S.	N.S.	*a*=0.59, ***b*=0.51**, *c*=0.27 $R^{2=}$0.58,AIC=-71.61
$Y=a+b^{*}$ N$+c^{*}$ P$+d^{*}$ LMA	*a*=-0.80 ***b*=0.79** ***c***=-0.02 *d*=0.09,R^2=0.40 AIC=-136.18	*a*=-0.29 *b*=0.34 ***c*=0.48** *d*=0.06,R^2=0.45 AIC=-60.86	*a*=-0.06 ***b*=0.58** *c*=0.07 *d*=0.01,R^2=0.13 AIC=-34.08	*a*=-1.79 ***b*=1.34** *c*=-0.23 *d*=-0.11,R^2=0.45 AIC=-41.70	*a*=-0.14,*b*=0.37, ***c***=0.11, ***d*=0.22,** R^2=0.59 AIC=-72.49

*Y represents either V_{cmax} or $J_{\max}$. n=46 for Rainy season, n=39 for transition and n=28 for Early dry season. n all data=113. Values in bold were more significant than the associated parameters in the same regression.

Table S 3. SMA slopes, 95% confidence intervals (in parenthesis) and coefficients of determination (R^2) for the relationship between photosynthetic traits (Y) and structural traits (X), across seasons. See Table 2 for abbreviations and units. Data for J_{max} is presented only for the Rainy Season.

Y	X	Rainy Season	Transition Rainy-Dry Season	Early Dry Season
V_{cmax_area}	J_{max_area}	0.97 (0.84,1.12),0.78	-	-
	N_{area}	1.41 (1.12,1.77),0.40 ^{a,A}	1.42 (1.06,1.91),0.20 ^{a,A}	1.35 (0.98,1.85),0.43 ^{a,A}
	P_{area}	1.17 (0.94,1.45),0.46 ^{a,A}	1.10 (0.80,1.50),0.10 ^{a,B}	NS
	LMA	0.66 (0.52,0.84),0.35	NS	NS
	R_{dark_area}	0.69 (0.50,0.95),0.24	NS	NS
	A_{sat_area}	1.02 (0.89,1.18),0.77 ^{a,A}	1.04 (0.80,1.36),0.34 ^{a,B}	NS
J_{max_area}	N_{area}	1.45 (1.17,1.79),0.49	-	-
	P_{area}	1.19 (0.98,1.46),0.54	-	-
	LMA	0.68 (0.55,0.84),0.52	-	-
	R_{dark_area}	0.70 (0.51,0.96),0.29	-	-
	A_{sat_area}	1.06 (0.86,1.28),0.55	-	-
A_{sat_area}	N_{area}	1.37 (1.07,1.76),0.28 ^{a,A}	NS	1.71 (1.25,2.33),0.43 ^{a,B}
	P_{area}	1.13 (0.89,1.43),0.34 ^{a,A}	NS	0.93 (0.65,1.33),0.26 ^{a,B}
	LMA	0.65 (0.49,0.83),0.22	NS	NS
R_{dark_area}	N_{area}	NS	1.24 (0.89,1.74),0.31 ^{a}	2.35 (1.82,3.03),0.62 ^{b}
	P_{area}	1.99 (1.43,2.77),0.22 ^{a}	0.97 (0.74,1.26),0.57 ^{b,A}	1.28 (0.98,1.69),0.58 ^{b,B}
V_{cmax_mass}	J_{max_mass}	1.17 (1.04,1.32),0.84	-	-
	N_{mass}	1.09 (0.91,1.31),0.61 ^{a,A}	1.23 (0.98,1.52),0.57 ^{a,A}	1.22 (1.04,1.44),0.85 ^{a,A}
	P_{mass}	1.01 (083,1.23),0.54 ^{a,A}	2.06 (1.56,2.73),0.26 ^{b}	1.05 (0.72,1.53),0.16 ^{a,A}
	SLA	0.81 (0.66,0.98),0.56 ^{a,A}	1.16 (0.92,1.47),0.51 ^{a,B}	1.01 (0.79,1.29),0.78 ^{a,AB}
	A_{sat_mass}	0.90 (081,1.01),0.87 ^{a,A}	0.96 (0.79,1.17),0.65 ^{a,B}	0.90 (0.69,1.17),0.59 ^{a,B}
J_{max_mass}	N_{mass}	0.93 (0.77,1.12),0.61	-	-
	P_{mass}	1.05 (0.86,1.27),0.57	-	-
	SLA	0.69 (0.56,0.84),0.53	-	-
	A_{sat_mass}	0.77 (0.65,0.90),0.72	-	-
A_{sat_mass}	N_{mass}	1.20 (1.00,1.44),0.61 ^{a,A}	1.26 (0.99,1.61),0.47 ^{a,B}	1.36 (1.13,1.62),0,82 ^{a,C}
	P_{mass}	1.36 (1.13,1.63),0.61 ^{a,A}	1.37 (1.06,1.75),0.42 ^{a,A}	1.25 (0.96,1.62),0.61 ^{a,B}
	SLA	1.72 (1.38,2.14),0.45 ^{a,A}	1.99 (1.55,2.55),0.45 ^{a,A}	2.13 (1.51,3.02),0.43 ^{a,B}

All bivariate relationships were considered significant at $p<0.05$. NS states a non-significant bivariate relationship. Slopes followed by the same lower case were not significantly different. Capital letters indicate differences on the elevation (intercept), evaluated only when there was no significant difference between slopes. Pairwise comparisons at $p<0.001$.

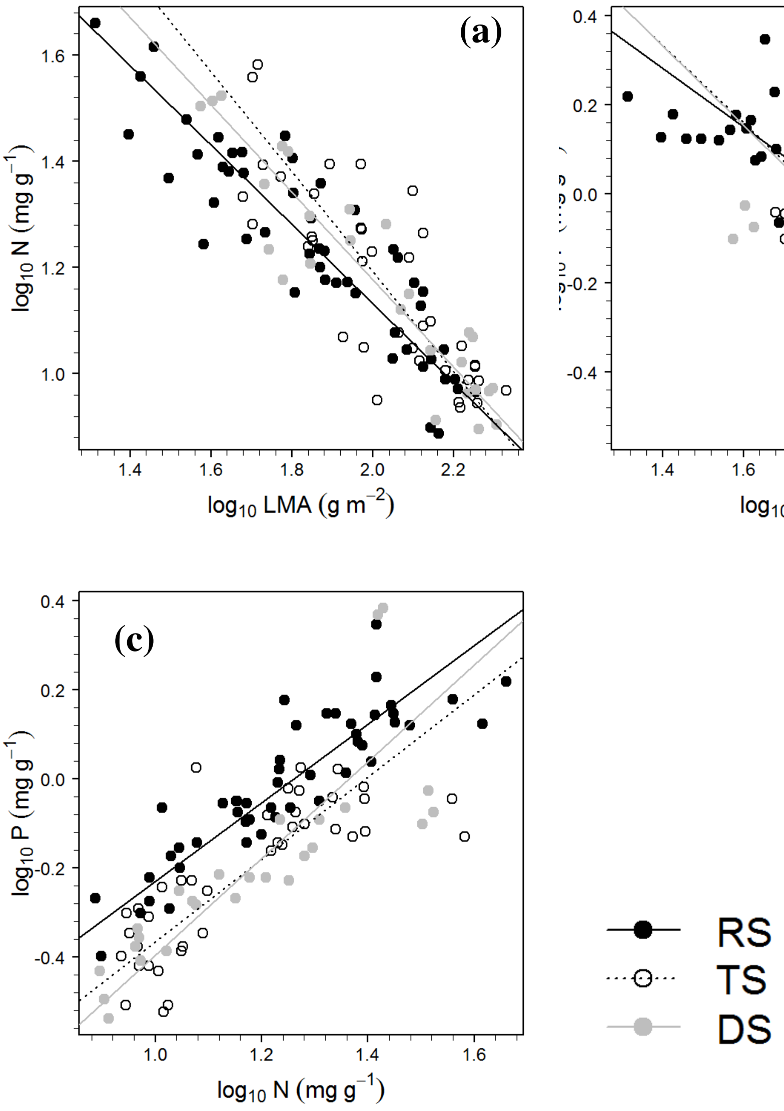

Figure S 3. Relationship between leaf mass per area (LMA), nitrogen (N) and phosphorus (P) content during the at the rainy season (RS), the transition to dry season (TS) and the early dry season (DS).All fits are standardized major axis (SMA) in log10 transformed data. All bivariate relationships were significant ($p<0.05$). **a)** There was not-significant differences between the SMA slopes (β) in the LMA-N relationship across seasons, $\beta=-0.78$, $R^2=0.75$. **b)** There was not-significant differences between the SMA slopes in the LMA-P relationship across seasons, $\beta=-0.83$, $R^2=0.59$. **c)** There was not-significant differences between the SMA slopes in the N-P relationship across seasons, but intercepts were significantly different between RS and the following seasons, $\beta=0.93$, $R^2=0.63$. All pairwise comparisons at $p<0.001$.

Table S 4. Partial correlation analysis for the relationship between V_{cmax}, J_{max} and the structural traits (N, P, LMA). Data is presented for V_{cmax} across seasons and as combined data set and for J_{max} during the rainy season only. All traits are considered on area basis.

	V_{cmax}				J_{max}
	All data	**Rainy Season**	**Transition**	**Early Dry Season**	**Rainy Season**
N	0.46*	0.20	0.33**	0.68**	0.24*
P	-0.02	0.29*	0.05	-0.30	0.23
LMA	0.12	0.08	0.01	-0.23	0.31**

Correlation for the paired comparison was significant**p<0.01 or marginally significant*p<0.05

Table S 5. Coefficients of determination (R^2) and Akaike Information Criterion (AIC) for the relationship between V_{cmax}, J_{max} and A_{sat} with stomatal conductance (g_S), leaf nitrogen content (N), leaf phosphorus content (P) and leaf mass per area (LMA). Values were the best fit in a stepwise regression. Data is presented for V_{cmax} and A_{sat} across seasons and as combined data set and for J_{max} during the rainy season only. All variables are expressed on an area basis and the data was transformed ($\log_{10}$) previous to the analysis.

	All data	**Rainy Season**	**Transition Rainy - Dry Season**	**Early Dry Season**
V_{cmax}	N_{area}, R^2=0.40, AIC=-138	g_S, N_{area}, P_{area}, R^2=0.57, AIC=-72	N_{area} R^2=0.17, AIC=-37	g_S, N_{area}, P_{area}, R^2=0.72, AIC=-59
J_{max}	-	P_{area}, LMA R^2=0.58, AIC=-72	-	-
A_{sat}	g_S, N_{area}, P_{area}, LMA R^2=0.73, AIC=-193.27	g_S, P_{area}, R^2=0.78, AIC=-107.34	g_S, N_{area}, LMA, R^2=0.49, AIC=-58.7	g_S, N_{area}, LMA, R^2=0.78, AIC=-52.7

Seasonal variation in J_{max}

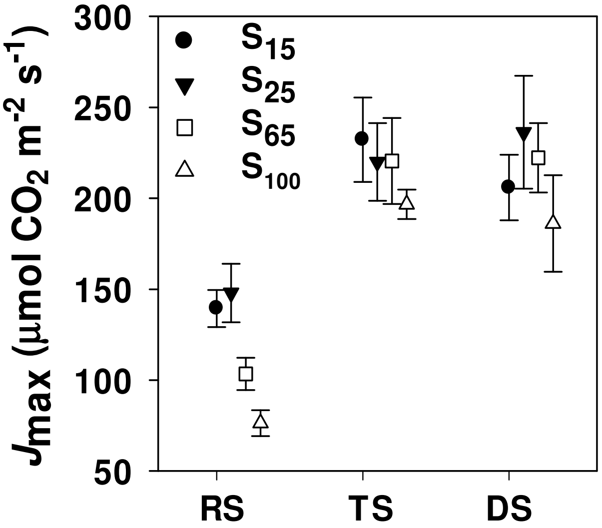

Figure S 4. Seasonal patterns of variation of the electron transport capacity (J_{max}). Each value is the average of the species average (n=4 per plot) in the rainy season (RS), the transition from rainy to dry season (TS) and the early dry season (DS).Vertical bars are standard errors. Symbols represent the forest sites (***S***) at the different regeneration ages (15, 25, 65 and >100 years since last disturbance). Values estimated in TS and the DS were discarded from all further analysis.

Respiration in the dark and Respiration derived from *A*/Ci curves

Respiration in the dark (R_{dark}) in µmol CO$_2$ m^{-2} s^{-1}was estimated for each leaf used for the *A*/Ci curve. Once the curve finished, the light in the cuvette was turned off and the cuvette was covered with aluminium foil for 5 minutes in the RS and at least 20 minutes in the following seasons. After that period, assimilation was recorded every 15 seconds and averaged over a 60 seconds period. Respiration measurements were normalized to 30 ºC using a Q_{10} response with a value of 2.2. (Tjoelker *et al.*, 2001). R_d (the dark respiration in the light), i.e. CO$_2$ evolution from mitochondria under light conditions was extracted from the *A*/Ci curves.

Although the average value of R_{dark} (2.00 ± 0.44 µmol CO$_2$ m^{-2} s^{-1}) decreased 40 % from the rainy season to the transition and then increased a 30% these differences were not statistically significant, perhaps because a smaller number of samples were used particularly for the first season. Additionally, the high values observed in the RS could be related to the post-illumination burst of CO$_2$ after the light was turned off (Noguchi, 2005), reason why the post-illumination time in the following seasons was extended.

R_{dark} (Figure S 5 a) was significantly different during the rainy season in both the early and the intermediated regeneration when compared to the mature forest where

R_{dark} was 70% lower. R_d was significantly different across seasons (Figure S 5 b), and it was significantly lower for all sites during the RS. However, not significant differences were found between sites within each season.

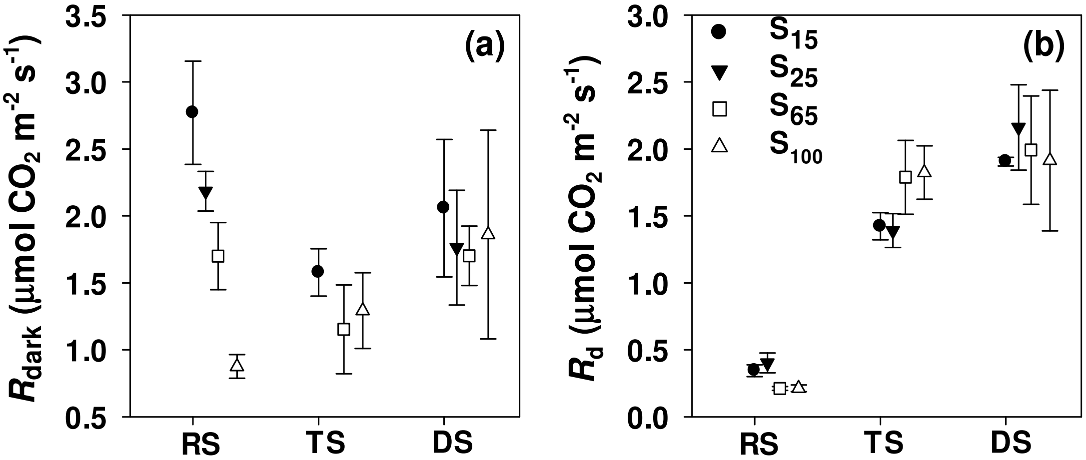

Figure S 5. Seasonal patterns of variation of **a)** Respiration estimated under dark conditions and **b)** CO$_2$ evolution from mitochondria under light conditions (R_d) extracted from the *A*/Ci curves. Each value is the average of the species average (n=4 per plot) in the rainy season (RS), the transition from rainy to dry season (TS) and the early dry season (DS).Vertical bars are standard errors. Symbols represent the forest sites (***S***) at the different regeneration ages (15, 25, 65 and >100 years since last disturbance).

Leaf thickness and leaf dry matter content

Leaf thickness was measured at three points per leaf using a thickness gage dial micrometre. When possible the thickness measurements were performed on sections of the leaf that excluded the midrib or major veins. Leaf thickness (Figure S 6 a) did not change seasonally for any of the sites, but important variations across the chronosequence were observed. In S_{15} was significantly higher (45%) than in S_{100} during the RS and DS. S_{15} and S_{25} were 30% higher than S_{100} during the TS and S_{15} was higher than S_{100} during DS. Not significant differences were observed with S_{65} or between the older or younger stands.

Seasonal changes in LDMC (Figure S 6 b) were not significant S_{15} and S_{100}. However, S_{25} presented a 30% difference between the RS and the DS. While S_{65}

presented a 25% increment from the RS to the TS and 40% the DS. Difference between sites within each season was not significant. The overall relationship of LMA and LMDC was 0.30 p<0.001.

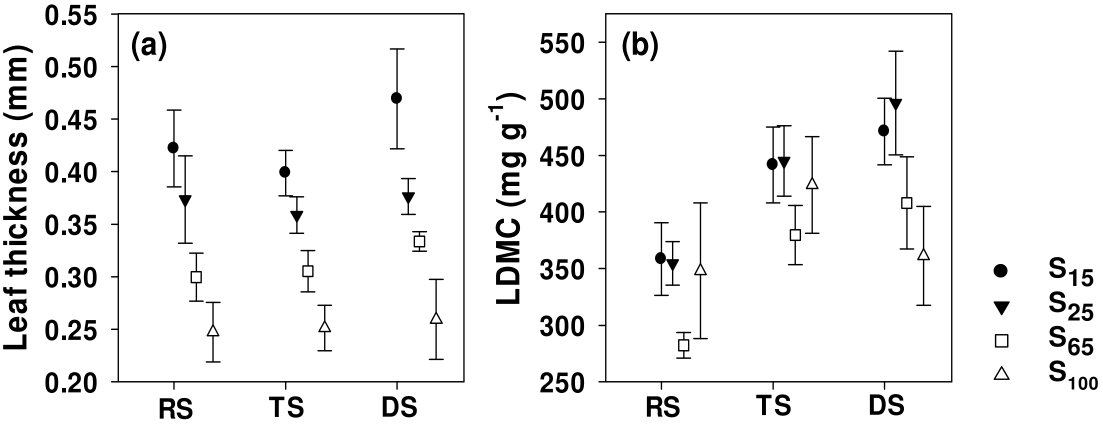

Figure S 6. Seasonal patterns of variation of **a)** Leaf thickness and **b)** Leaf dry matter content. Each value is the average of the species average (n=4 per plot) in the rainy season (RS), the transition from rainy to dry season (TS) and the early dry season (DS).Vertical bars are standard errors. Symbols represent the forest sites (***S***) at the different regeneration ages (15, 25, 65 and >100 years since last disturbance).

Variation on V_{cmax}, J_{max}, A_{sat}, N_{area} and P_{area} at species level.

Table S 6. Seasonal patterns of variation of photosynthetic and structural traits for the four species present in at least three sites along the chronosequence. Symbol + indicate if the parameter presented significant seasonal variation and the accronyms following the symbol indicate the season (Rainy [RS], Transition [TS] or Early Dry seasons [DS]) were the maximum and significantly different value was measured. The symbol = indicates no significant change accross seasons when compared with pairwise t test (with Bonferroni correction). n.a. indicates insuficient data for statistical comparison

Species	**Site**	V_{cmax}	A_{sat}	N_{area}	P_{area}	**LMA**
Redhera trinervis	S_{15}	+TS	=	+DS	+RS	+TS,DS
	S_{25}	=	+ RS	=	+RS	+TS,DS
	S_{65}	+TS	=	=	=	+RS
Cochlospermum vitifolium	S_{15}	=	+RS	=	+RS	+RS
	S_{25}	n.a.	n.a.	=	=	+TS
	S_{65}	=	=	=	=	+TS
Semialarium mexicanum	S_{15}	=	=	+DS	+DS,RS	+DS
	S_{25}	+TS	+RS	=	+RS	=
	S_{65}	=	=	=	=	=
Luehea candida	S_{25}	n.a.	n.a.	=	=	=
	S_{65}	=	=	+TS,DS	=	+TS,DS
	S_{100}	=	+TS	=	=	+TS,DS

Table S 7. Summary of the analysis of variance exploring seasonal variability, and across the chronosequence. The asterisks indicate if the seasonal or variation across the chronosequence was significant. N.S. not significant.

Species	**Site**	V_{cmax}	A_{sat}	N_{area}	P_{area}	**LMA**
Redhera trinervis	Chronosequence	N.S.	N.S.	N.S.	**	***
	Seasonal	**	**	*	*	***
Cochlospermum vitifolium	Chronosequence	N.S.	N.S.	*	N.S.	*
	Seasonal	N.S.	N.S.	N.S.	**	***
Semialarium mexicanum	Chronosequence	N.S.	N.S.	N.S.	N.S.	***
	Seasonal	***	*	**	N.S.	**
Luehea candida	Chronosequence	*	*	*	N.S.	***
	Seasonal	N.S.	*	**	N.S.	***

***p<0.001,**p<0.01,*p<0.05

Table S 8a. Mean values of maximum carboxylation capacity (V_{cmax}, µmol CO_2 m^{-2} s^{-1}), maximum rate of electron transport (J_{max}, µmol CO_2 m^{-2} s^{-1}), assimilation rate at light saturating conditions (A_{sat}, µmol CO_2 m^{-2} s^{-1}) and stomatal conductance at which A_{sat} was determined (g_S, mol H_2O m^{-2} s^{-1}). Values are the average and standard deviation for each species sampled during the rainy season (RS), the transition into dry season (TS) and the early dry season (DS). Sample size (n) includes measurements across different sites in the chronosequence (except for *B.crassifolia* and *M.chicle* that were only measured on one site).

Species	**Field season**	V_{cmax}	J_{max}	A_{sat}	g_S	**n**
Byrsonima crassifolia	RS	65.9 ± 6.2	140.0 ± 17.1	11.3 ± 0.1	0.25 ± 0.05	2
	TS	114.6 ± 22.7	272.0 ± 30.8	8.2 ± 0.8	0.13 ± 0.04	4
	DS	59.7	177.7	2.6	0.07	1
Cochlospermum vitifolium	RS	77.2 ± 7.6	138.1 ± 10.3	13.4 ± 1.4	0.28 ± 0.05	9
	TS	83.7 ± 16.2	212.0 ± 23.3	9.4 ± 2.0	0.18 ± 0.02	5
Exostema mexicanum	RS	33.8 ± 2.0	62.9 ± 5.9	6.9 ± 1.1	0.24 ± 0.09	4
	TS	76.3 ± 0.0	212.0 ± 20.6	5.0 ± 0.6	0.07 ± 0.00	2
	DS	81.8 ± 9.1	216.6 ± 22.5	4.7 ± 1.5	0.08 ± 0.02	3
Hymenaea courbaril	RS	49.4 ± 6.2	68.0 ± 6.9	9.3 ± 1.6	0.19 ± 0.02	2
	TS	61.1 ± 2.8	193.7 ± 8.2	5.9 ± 0.5	0.10 ± 0.01	3
	DS	70.1 ± 6.2	208.6 ± 3.4	7.3 ± 0.5	0.10 ± 0.02	2
Luehea candida	RS	51.8 ± 3.6	86.7 ± 7.1	10.0 ± 0.8	0.30 ± 0.08	11
	TS	54.7 ± 2.0	177.7 ± 5.3	7.1 ± 0.7	0.16 ± 0.02	5
	DS	52.1 ± 3.8	167.4 ± 10.9	5.0 ± 0.8	0.11 ± 0.02	6
Redhera trinervis	RS	85.4 ± 7.8	152.3 ± 13.3	14.2 ± 1.4	0.29 ± 0.04	10
	TS	115.1 ± 10.3	263.1 ± 13.2	10.0 ± 1.0	0.17 ± 0.02	11
	DS	73.5 ± 6.0	218.9 ± 13.8	5.5 ± 0.8	0.11 ± 0.02	6
Semialarium mexicanum	RS	52.3 ± 3.7	114.5 ± 8.9	9.2 ± 0.6	0.19 ± 0.02	9
	TS	74.0 ± 5.4	212.0 ± 7.3	6.6 ± 0.5	0.13 ± 0.01	9
	DS	96.5 ± 9.3	258.3 ± 12.6	6.5 ± 0.6	0.12 ± 0.01	8
Manilkara chicle	RS	43.14	95.43	7.45	0.11	1

Table S 8b. Mean values of leaf nitrogen content (N_{mass}, mg g^{-1}), leaf phosphorus content (P_{mass}, mg g^{-1}), leaf mass per area (LMA, g m^{2}), leaf thickness (mm), leaf dry matter content (LDMC, mg g^{-1}) and N:P ratio. Values are the average and standard deviation for each species sampled during the rainy season (RS), the transition into dry season (TS) and the early dry season (DS). Sample size (n) includes measurements across different sites in the chronosequence (except for *B. crassifolia* and *M. chicle* that were only measured on one site)

Species	Field Season	N_{mass}	P_{mass}	LMA	Leaf thickness	LDMC	N:P	n
Byrsonima crassifolia	RS	9.14 ± 0.80	0.47 ± 0.03	139.8 ± 0.4	0.40 ± 0.01	452 ± 7	19.6 ± 0.8	3
	TS	11.00 ± 0.49	0.38 ± 0.03	147.6 ± 12.0	0.40 ± 0.03	452 ± 2	29.1 ± 1.8	4
	DS	9.06 ± 0.34	0.32 ± 0.01	157.1 ± 20.7	0.40 ± 0.01	476 ± 5	28.6 ± 1.0	4
Cochlospermum vitifolium	RS	20.60 ± 1.44	1.24 ± 0.13	67.5 ± 3.9	0.31 ± 0.02	318 ± 4	17.2 ± 0.9	11
	TS	15.02 ± 1.10	0.70 ± 0.06	92.2 ± 2.9	0.31 ± 0.02	389 ± 2	21.8 ± 0.7	12
Exostema mexicanum	RS	37.44 ± 3.66	1.43 ± 0.08	27.1 ± 3.0	0.19 ± 0.01	210 ± 7	26.2 ± 1.9	4
	TS	38.24 ± 0.85	0.89 ± 0.08	46.6 ± 2.8	0.19 ± 0.01	319 ± 4	43.0 ± 4.4	3
	DS	32.62 ± 0.43	0.86 ± 0.04	40.0 ± 1.4	0.15 ± 0.02	287 ± 3	38.3 ± 1.8	3
Hymenaea courbaril	RS	21.07 ± 1.76	1.08 ± 0.03	71.4 ± 3.9	0.21 ± 0.01	419 ± 12	19.6 ± 1.4	4
	TS	17.22 ± 0.48	0.78 ± 0.04	120.3 ± 6.8	0.25 ± 0.02	529 ± 4	22.3 ± 0.8	5
	DS	25.53 ± 2.15	2.40 ± 0.22	62.0 ± 5.3	0.27 ± 0.02	300 ± 9	10.7 ± 0.5	5
Luehea candida	RS	21.77 ± 1.52	1.20 ± 0.08	46.2 ± 4.1	0.28 ± 0.01	587 ± 21	18.3 ± 0.9	11
	TS	20.73 ± 1.14	0.81 ± 0.04	66.0 ± 4.9	0.32 ± 0.01	456 ± 4	23.8 ± 2.4	12
	DS	17.74 ± 1.00	0.68 ± 0.05	73.2 ± 5.1	0.33 ± 0.01	496 ± 4	26.4 ± 1.0	12
Manilkara chicle	RS	16.97 ± 0.45	0.79 ± 0.08	108.9 ± 6.5	0.30 ± 0.01	415 ± 9	21.9 ± 2.7	2
	TS	17.83 ± 1.06	0.73 ± 0.06	114.1 ± 7.2	0.27 ± 0.01	423 ± 3	24.6 ± 0.5	4
	DS	19.61 ± 1.03	0.85 ± 0.08	99.1 ± 11.0	0.30 ± 0.05	381 ± 10	23.2 ± 1.3	3
Redhera trinervis	RS	14.62 ± 1.26	0.87 ± 0.05	112.6 ± 9.7	0.42 ± 0.02	296 ± 6	16.8 ± 0.8	13
	TS	14.01 ± 1.70	0.58 ± 0.05	148.3 ± 14.1	0.37 ± 0.02	457 ± 10	23.7 ± 1.3	13
	DS	12.46 ± 1.59	0.51 ± 0.06	157.6 ± 12.8	0.40 ± 0.02	492 ± 11	24.4 ± 1.1	10
Semialarium mexicanum	RS	12.10 ± 1.07	0.71 ± 0.08	122.0 ± 11.0	0.44 ± 0.03	329 ± 4	17.5 ± 0.8	10
	TS	14.39 ± 1.15	0.66 ± 0.08	130.6 ± 8.5	0.38 ± 0.02	383 ± 2	23.9 ± 1.6	13
	DS	13.61 ± 1.41	0.56 ± 0.05	149.4 ± 14.5	0.44 ± 0.03	390 ± 4	23.8 ± 0.9	10

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Chapter 5. Discussion and general conclusions

Discussion

The main objective of this thesis was to enhance our understanding of the implications that the co-existence of species and their individual traits and needs for the use of resources has over ecosystem productivity and nutrient cycling. Additionally, we tried to identify how these traits could be linked to ecosystems' responses to climatic factors.

We used two resource-limited ecosystems, a grassland in a temperate region and a seasonally dry tropical forest, to address our main objective. Despite the differences between the two ecosystems in term or their physiognomy, species composition and metabolic paths for carbon allocation; they are both characterised by the existence of pulses of water and nutrients, high species diversity and limiting conditions to growth (water and phosphorus limitation in particular). Hence we found some common questions and mechanisms relative to both that will be also addressed on this discussion.

We started our research with three main questions:

1. What are the linkages between leaf traits and community functioning/productivity in a grassland subjected to artificial climate change?
2. How is gross primary productivity and ecosystem respiration of a limestone grassland affected by imposed climatic manipulations?
3. What are the main controls of the temporal/spatial variation in photosynthetic efficiency of dominant species in a seasonally dry tropical forest?

And our general conclusions related to each of them were:

1. Leaf traits can effectively be aggregated either as a weighted mean or as an emergent property to characterise the effects of the main treatments on productivity. However, it is relevant to consider not only the identity (the specific trait), but also its relative abundance. Changes in species abundance across the experiment (as species composition was quite similar), was the most determinant factor of the differences observed in productivity (explored as total foliar nitrogen).

2. Changes in water availability have caused important changes in gross primary productivity (GPP) and ecosystem respiration. These changes are controlled by the interaction of soil moisture with above ground biomass, but there is also an important effect of the conditions experienced by the ecosystem prior to the measurement, which particularly affected ecosystem respiration in the control plots. Despite the differences observed in GPP, we suggest that the stability found in the biomass harvest is likely explained by higher allocation belowground structures and the reduction of the leaf area when water availability is limited (the drought plot) and a faster leaf turnover when water is in excess (watered treatment).
3. Photosynthetic efficiency parameters (V_{cmax} and J_{max}), are strongly linked to leaf nitrogen and phosphorus content, particularly during the rainy season. However, there is a strong seasonal pattern on this relationship, most likely associated to changes in N:P ratio by the mobilisation of phosphorus. We found that assimilation at saturating light and ambient CO_2 is more strongly controlled by changes in stomatal conductance, rather than by changes in N. Seasonal changes in resources seem to have a larger impact on the photosynthetic parameters than resource changes along the succession gradient. At species level, values of photosynthetic traits are more constrained than values of structural traits.

As stated in the introduction, leaf traits can be used at individual level (i.e. species level) and can also be aggregated at different levels to answer questions that address immediate and long-term responses to our changing climate. Therefore I have framed my final discussion around how these levels of aggregation help answering our research questions. Additionally, I discuss issues that emerged from this final analysis, including technical limitations of our approaches, and propose new questions for future research.

Leaf traits and implications for nutrient cycling

In Chapters 2 and 4 we addressed how the particular properties of the species under study could influence nutrient cycling in both ecosystems. The species studied in Buxton are characterised by high leaf mass per area (LMA), particularly in the drought plot, and by relatively high C:N ratios (average 33.4 ± 0.8 in the treatments). Therefore we suggested that the material is highly recalcitrant and has a direct impact on the fragmentation of the materials and the activity of the decomposers (Grime *et al.*, 1996). The species studied in the SDTF were also characterised by high LMA and by relatively high C:N ratios particularly in the transition and the early dry season (average 31 ± 1 and 33 ± 2 respectively).

Across both sites high LMA and lower C:N ratios have important implications for nutrient cycling, especially considering that the C:N ratio could be significantly higher towards the end of the leaf life span, where more N could be removed before leaf shedding (McGroddy *et al.*, 2004). This is particularly relevant in the SDTF, where important N remobilisation rates have been reported (Lal *et al.*, 2001). It is clear that the technical approach used in Buxton, allows us to address the effects of leaf traits properties on nutrient cycling with more confidence. However, as we used the dominant species in the SDTF it is likely that they can also be used to study the prevailing dynamics in this ecosystem. From the analysis of leaf traits and strategies across the two sites three questions emerged:

1. How are leaf mass per area (LMA) and Leaf Nitrogen Content (LNC) changing across both ecosystems? It is there a common axis of their relationship?

The range of LMA and LNC values for both ecosystems was comparable and presented a relatively similar level of variability, particularly for LMA. LMA in the grasslands varied between 14.1 and 164.3 g m^{-2} and between 20.66 and 228.07 g m^{-2} in the SDTF with an overall coefficient of variation (CV) of ~40%. A high inter-specific variability was observed in LMA and, although we have a limited number of within-species repetitions in both sites, it is likely that the high variability reflects the plasticity of this trait particularly in response to light (Poorter *et al.*, 2009). Variability on LNC in Buxton (0.73-3.16 % N, CV ~31%) was more constrained that in the SDTF (0.8-4.6 % n, CV ~40%), which is perhaps related to the seasonal variation captured in the SDTF but also to the smaller number of samples used in the STDF.

Both data sets showed that in general species at the lower range of the specific leaf area (or high LMA) tend to have lower N, as predicted by Wright *et al.* (2004). However, despite the similarities observed in LMA and LNC across both ecosystems, the bivariate relationship between both traits in the grasslands (Chapter 2) was not as significant as in the SDTF (Chapter 4 supplementary material). The LMA-LNC relationships represents the existence of a trade-off between allocation to structural tissues versus other processes (Shipley *et al.*, 2006), and it is likely that it could also represent differences in allocation even within the same leaf (particularly in the grasslands). For the weaker LMA-LNC observed in Buxton, in comparison with the STDF, I suggest two possible explanations. First, for our estimation of the leaf nitrogen content in Buxton, we used leaves from different sections of the selected plant (as our main interest was to characterise nitrogen availability at canopy level), so our results could simply reflect different LMA-LNC relationships within the same species/plant. Second, that our results could be affected by the inclusion of other non-leaf material (i.e. photosynthetic stems) where the expected LMA-LNC relationship for leaf traits for is not so clear.

2. How does our leaf trait data compare to the global context?

This question emerged linked to the previous question and to the fact that the existence of an important number of trait data sets (i.e. TRY, GLOPNET), and has allowed exploration of more general relationships, particularly between structural investment (LMA), photosynthetic rate, leaf life span and nitrogen content.

To set our data in this global context, we compared the slope of the LMA-LNC relationship from Buxton, and from the SDTF with the slope for this relationship reported by Wright *et al*. (2004). The slopes across the three data sets (Figure 1) were not significantly different although the significance of this relationship in the grasslands, as thoroughly discussed in Chapter 2, is very low (R^2=0.1), compared to the SDTF ($R^{2=}$0.72) or with the global data set (R^2=0.57).

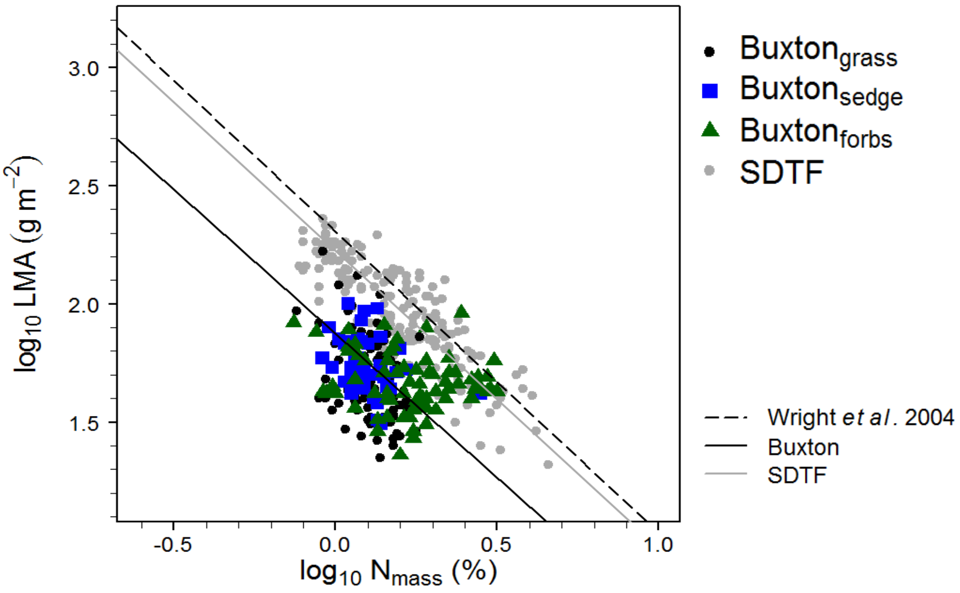

Figure 1. Relationship between leaf mass per area (LMA) and leaf nitrogen content (N_{mass}) for the species studied in the seasonal dry tropical forest (SDTF) and for the species studied in Buxton partitioned by functional type. Axes are in $\log_{10}$ scale. The full lines refer to standardized major axis (SMA) line-fitting applied to each data set and the solid line is the SMA fit from the global data set presented by Wright *et al.* (2004). There was no significant difference between slopes ($p<0.05$).

The differences for the significance of the bivariate relationships could be caused by the clear differences observed in this relationship across functional types in Buxton (Figure 2 Chapter 2), and the methodological issues discussed in the previous section. Additionally, by fact that this global data set (Wright *et al.*, 2004), is still largely dominated by C_3 plants, and important differences have been observed for grassland species compared in this context due to differences in their metabolic pathways (Feng and Dietze, 2013). However, as the leaf traits data bases are constantly growing it would be interesting to analyze this patterns looking at functional types/metabolic pathways in more detail.

3. How could the grassland ecosystem be affected by the P-limitation?

This question emerged from our results in the SDTF where we found our photosynthetic parameters (V_{cmax}, J_{max} and A_{sat}) were related, at least in the rainy season, not only to nitrogen content but also to phosphorus. From our results, consistent with other results from the tropics (Domingues *et al.*, 2010, Meir *et al.*, 2007), we suggested that species in P-limited ecosystems, like the ones under study (Vitousek *et al.*, 2010, Grime and Curtis, 1976), are likely to be more efficient in the remobilisation of P, which is relevant in the nutrient recycling context.

We do not have information on photosynthetic efficiency at leaf level for this grassland ecosystem, although at the moment this information is being collected under controlled growth conditions (Fridley J.D. pers. comm.). It would be interesting to explore if the species in Buxton, whose leaf traits already suggest they are stress adapted plants (Grime *et al.*, 2008), could show a similar pattern, particularly under field conditions, to the one we observed in the SDTF in terms of the N or P limitation.

Ecosystem emergent properties, applications and limitations

In Chapters 3 and 4 we explored the use of emergent properties (properties of an entire system *sensu* Salt (1979)), and we used them to characterise nutrient availability (total foliar nitrogen, g of N m^{-2} ground) in the grasslands and canopy structure (leaf area index, m^2 leaf area m^{-2} ground) in both ecosystems. Both properties have shown to be strongly linked to gross primary productivity (Williams and Rastetter, 1999, van Wijk *et al.*, 2005), and therefore are widely used to scale from leaf to canopy/ecosystem at field scale, as we did, or through modelling.

In Buxton, the destructive harvesting and LAI estimation showed that, although the employed techniques to characterise the aboveground biomass properties were not ideal to estimate LAI, they were useful to at least categorise our microsites in terms of aboveground biomass (Figure 2). However, the underestimation in the LAI from the indirect technique (compared to the destructive estimation) certainly limited the use of this relationship to normalise our GPP data (Chapter 3) by leaf surface area as it is usually done for these type of studies (van Wijk and Williams, 2005, Street *et al.*, 2007, Shaver *et al.*, 2007).

Figure 2. Aerial view of microsites in Buxton. Estimated values of Leaf Area Index: Control=3.1 $m^2 m^{-2}$, Watered=3.3 $m^2 m^{-2}$, Drought=0.9 $m^2 m^{-2}$, Heated=3.1 $m^2 m^{-2}$. NDVI=0.87 for all sites. Photos taken in June 2010.

In the SDTF, we estimated plant area index (PLAI, the contribution of LAI and stems and branches) and we used the results to characterise canopy openness and seasonal changes in light availability (Figure 2, Chapter 4). Our data was consistent with previous reports from the site (Kalacska *et al*., 2005) that showed 40% higher PLAI in the late forest and significant seasonal variation in the early stages related to the dominance of deciduous species. However, it is clear from our results that the estimation of PLAI from indirect methods in open areas is inaccurate; particularly in the early dry season when the leaf shedding was evident but no significant difference was observed on the PLAI.

Although the existence of indirect methods to estimate canopy structure has been an important advancement in the ecological field (Jonckheere *et al*., 2004), we found limitations in their use, so we explored possible alternatives or additions to the approaches used. Clearly it is beyond the scope of this thesis to address any method in detail but it is possible to point out a few potential improvements to the methodology used. The question that emerged from this section was:

How can we improve our LAI estimations?

In the grasslands two main complications emerged. First, the high species density and richness limited the accurate use of point contact methods or the use of the vegetation index (NDVI). Second, the short stature limited the use of gap light methods. The possible approaches that could be suggested include the use of downward facing photography (digital images), either normal or hemispherical, that can be converted through mathematical algorithms in leaf area index (Cescatti, 2007). Although this methodology is still being improved, it has given good results on the estimation of plant cover on short canopies and crops (Liu and Pattey, 2010, Meyer and Neto, 2008). However, as the technique depends on the spectral separability between vegetation and soil background (Przeszlowska *et al.*, 2006), it could be equally limited at high LAI values. Another approach is that biomass could be estimated using a falling plate or disc (Dörgeloh, 2002) calibrated against biomass estimations. To avoid more destructive harvesting, this calibration could be performed just before the artificial grazing in October. However, the issue of the steppe slope and how this would affect the way the plate would be seated on the surface should be considered.

The PLAI estimation in the SDTF, particularly in the early forest stages, could be improved through the use of allometric techniques, using the relationship between leaf area and the woody elements carrying that green biomass (Jonckheere *et al.*, 2004). Additionally if the main interest is to estimate only LAI (i.e. to calculate total foliar nitrogen), the correction of the PLAI suggested by Kalacska *et al.* (2005), using the PLAI measurements taken during the dry season to correct the measurements during the rest of the year, could be a suitable approach to improve LAI estimation.

Seasonal and spatial variability on productivity: from leaf to ecosystem level

Gross primary productivity (GPP) is not only the primary measure of carbon supply but also represents metabolic activity in the canopy (Malhi, 2012). As such, capturing its seasonal and spatial variability and the main drivers of its change is of relevance to understand long-term responses to changes in resource availability and climatic constrains. As such, two questions emerged from this section that I address independently for each site:

1. Can we address seasonal variation of GPP in the grasslands?

In the grasslands (Chapter 3), we found that spatial variation on soil moisture and above ground biomass was important to understand the observed changes in GPP across the ecosystem. In Chapter 3 we also proposed two mechanisms that could explain why, despite significant differences observed in our GPP measurements, NPP (standing biomass at the end of the year) was relatively stable. We suggested that the stability of the NPP might be explained in the drought plot by a higher allocation to below ground structures and the reduction of the leaf area index to withstand water stress. By contrast, in the watered treatment stable NPP could be explained by a rapid leaf turnover. Additionally, as it has been observed that the winter-warming advances the growing season (Grime *et al.*, 2008), we proposed that the treatment is likely to alter the time when the ecosystem changes from CO_2 sink to source, as observed by Saleska *et al.* (1999) in a similar experiment, but not the annual carbon budget. This would likely explain the no-difference observed on GPP or R_{eco} between this treatment and the control.

Therefore, I suggest that two possible approaches could contribute to improve our understanding of the seasonal variations on GPP on this grassland. First, flux measurements could be carried out over the entire growing cycle which would allow for constructing a carbon budget for the ecosystem and to capture the sink/source (Loik *et al.*, 2000, Saleska *et al.*, 1999, Niu *et al.*, 2008). However, this would require an important time investment to capture not only seasonal/spatial patterns, but also diurnal cycles of variation. Second, we could use process-based modeling to upscale the ecosystem CO_2 fluxes measured here and to incorporate other environmental drivers to help clarify GPP variation (Shaver *et al.*, 2007, Williams and Rastetter, 1999, Williams *et al.*, 2006). Additionally the incorporation of phenological responses of plants during the growing stage, could also help in the interpretation of the timing and magnitude of the carbon exchanges (Sus *et al.*, 2010). Perhaps the main limitation of this approach is that the ecosystem is usually treated as a single functional type; we have made it clear that species composition/abundance in Buxton is not uniform and implications related to the inclusion of treatment/species-specific parameters that modify the TFN-LAI relationship (Street *et al.*, 2012), and the role that more diverse communities have on the GPP-LAI relationship (Fletcher *et al.*, 2012), should therefore be considered. This adds to the previously discussed need of improving the indirect estimation the LAI.

2. Could we predict productivity at ecosystem level in the SDTF?

In the dry forest four important conclusions that emerged from our leaf level analysis (Chapter 4) have important implications for up-scaling purposes. First, species in both the early and late stages of the forest tend to have a similar set of traits; however, important differences were observed between S_{25} and S_{65}, perhaps related to the effect of species composition on soil properties, particularly in terms of water availability and hydraulic conductivity (Leiva *et al.*, 2009). Second, our data corroborates that the stomatal conductance (g_S) plays an important role in defining observed carbon exchange strategies in the forest. However, it was also clear that the control of g_S over A_{sat} was stronger, or happens earlier, than over V_{cmax}, as previously reported for drought environments (Flexas and Medrano, 2002). Third, our results showed that the J_{max}:V_{cmax} ratio is not constant across the chronosequence and, although we could not explore the seasonality of this relationship, it is likely it could be affected by the response of J_{max}, in particular, to seasonal changes in light and water availability (Wright *et al.*, 2013) or even temperature (Hikosaka *et al.*, 2006). Fourth, our results showed both the biochemical parameters (V_{cmax} and J_{max}) are affected not only by nitrogen availability but also by phosphorus availability, particularly on the onset of the growing period.

It is clear our plot level averages only provide an estimation of what is happening with the dominant species, and that to generalise these important results to landscape level we should be integrating species responses across different spatial/temporal scales. Although modelling gross primary productivity in the SDTF requires a large level of detail (Meir and Pennington, 2011), it is certainly necessary to understand the implications that inter-annual or long-term variability in precipitation and temperature could cause in the recovery of this ecosystem (Enquist and Enquist, 2011).

Although an important number of vegetation or ecosystem process models have been developed over the last decades (Ostle *et al.*, 2009), in the context of this thesis and the information collected here, I would suggest to approach the up-scaling need by using the soil-plant-atmosphere (SPA) model developed by Williams *et al.*(1996), primarily because SPA explains carbon fluxes in terms of optimal water use, which is important for the SDTF where the dynamic changes in the efficiency of water carriage through the leaf profoundly affect leaf gas exchange (Brodribb and Holbrook, 2007).

SPA has successfully been used to describe drought effects on forest productivity (Fisher *et al.*, 2008, Whitley *et al.*, 2011, Zeppel *et al.*, 2008), and recently Wright *et al.* (2013) showed that, calibrated with leaf level measurements (like the ones we gathered in our study) and linked to stand structure, the model is able to explain the differences in ecosystem production, particularly across a soil moisture gradient. Furthermore, as SPA has a detailed soil profile, it could be useful to capture changes in soil hydraulic conductivity/water retention evident with succession age (Leiva *et al.*, 2009), and the seasonal variation in soil moisture that happens rapidly in the top layers once rain stops (Fallas-Cedeno *et al.*, 2010, Rojas-Jimenez *et al.*, 2007).

However, in this modeling context additional challenges should be considered, beside the fine model-tuning needed. First, the SDTF is characterised by an asynchronous canopy flushing, so leaf age and time during the year can usually not be treated as synonymous (Kitajima *et al.*, 1997). Additionally, this would imply that ecosystem responses will be the combination of trees with leaves of different ages and with differential abilities to respond to environmental stresses (Reich, 1995, Reich and Borchert, 1988). Second, the previously discussed limitations on the LAI estimation should be resolved, especially since LAI (representing light interception area) is one of the main factors considered to accurately estimate GPP at stand level (Whitley *et al.*, 2011). Third, at the moment there are no flux towers at this site (AmeriFlux, 2013), which does not allow for a top-down estimation of GPP (Malhi, 2012), which could be useful to validate model estimations. So this corroboration should possibly be done at leaf level (Wright *et al.*, 2013), or eventually at plant level (Zeppel *et al.*, 2008), if sap-flow data becomes available (Powers J.S. pers. comm.).

Concluding remarks

We have learned that leaf traits provide important information of species strategies in the use of resources and that they can be linked through different means (aggregation, as emergent properties and through modelling processes) to address questions of time and scale.

It is clear that research is always limited in time and space, and short-term studies about ecosystem dynamics are not always ideal to help understand the factors controlling processes in time. This caveat notwithstanding, our study provides the first opportunity to explore the effect of a long-term climatic manipulation on the gross primary productivity (GPP) of this limestone grassland. Additionally, we have provided the first set of photosynthetic efficiency traits for the SDTF in Costa Rica, which could be eventually used to predict GPP over larger scales with important implications for management and conservations purposes.

From the experiment in Buxton we have learned that long-term studies can help to deepen our understanding of process-level mechanisms. We have also learned that field-scale manipulations are an important tool for understanding possible responses to changes in biosphere biochemistry, but that these responses are not unidirectional, particularly because we cannot address to what extent the plant-soil interactions are being affected by other factors (i.e. soil fertility).

From our research in the SDTF we have learned that species have different strategies in the use of resources and that these strategies present a strong seasonal variation. Additionally, we found that phosphorus is also a limiting factor to photosynthetic efficiency and therefore its role on gross primary productivity at ecosystem level should be explored.

From both ecosystems we have learned that scale is important if we aim to predict ecosystem response to changes in resources, and that some other factors could be explored to improve the understanding of the carbon cycling in this ecosystem, i.e. soil respiration, microorganism's response to the treatments and other nutrient limitations to photosynthesis.

From this work I have learned that although doing research at different scales and in different ecosystems was challenging, it is also a unique opportunity to widen one's knowledge, to interact with researchers in different fields and to generate ideas for future research.

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