Intraspecific variation in Leaf traits of Tropical trees

Effect of light and climate

Thesis submitted in partial fulfillment of the requirements of Five Year BS-MS Dual Degree Program

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By

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Certificate

This is to certify that this dissertation entitled "Intraspecific variation in leaf traits of tropical trees: Effect of light and climate" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by Neha M of IISER Pune under the supervision of Dr. Mahesh Sankaran, Ecology and Evolution, NCBS during the academic year 2014-2015.

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Declaration

I hereby declare that the matter embodied in the report entitled "Intraspecific variation in leaf traits of tropical trees: Effect of light and climate" are the results of the investigations carried out by me at the Department of Biology, NCBS-Bangalore and IISER-Pune, under the supervision of Dr. Mahesh Sankaran and Dr. Deepak Barua, and the same has not been submitted elsewhere for any other degree.

Neha M

Abstract

Functional traits are easily quantifiable variables that help in understanding species distribution patterns and their responses to changes in the environment. The consequence of intraspecific variation in leaf functional traits in understanding these patterns and their importance in community assembly and climate change models has only recently been recognized. This study examined the effect of climate and light on five leaf functional traits in 29 species across three sites, each hundreds of kilometers apart from the other, in the poorly understood dry tropical forests of India. There was significant interspecific and intraspecific variation for all traits across all three sites. The direction of variation in traits was consistent with the existing literature. In addition, seasonality of rainfall was found to be more important than total rainfall in determining the intraspecific trait variation between sites. Plasticity of traits due to light were dependent on species and site identities. There was no relation between plasticity due to light and inter-site variation. Further studies are required to understand the mechanistic causes of these larger scale patterns.

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Introduction:

Plant functional traits are defined as morphological, physiological or phenological measurements of an individual, without any reference to the environment or level of organization that affects the performance of the individual and therefore, indirectly affects the fitness of the plant (Violle et al., 2007). These traits become very important in understanding nature as described by Schimper (1898). In Schimper's world, an organism's performance is dependent on the physical features that they possess which is independent of their species identity (Westoby and Wright, 2006). For decades, people have studied traits as a connective link between physiology and performance of the plants, across different experimental and environmental conditions (Poorter and Bongers, 2006). It was speculated that these traits would help is understanding species tolerance levels and distribution patterns at the community level. In the recent past, they have been shown to determine the success and function of plant in any habitat (Westoby et al., 2002). Functional traits help to describe the ecology of species with a few easily quantifiable variables. They are very useful in studying species distribution patterns as pointed to by a growing number of studies showing that functional traits are strongly correlated to the habitat of the individual (Ordoñez et al., 2009; Reich et al., 1999; Wright et al., 2004, 2005). Functional diversity defined by the distribution of traits in a community is finding application in community assembly models to tease apart the effects of habitat filtering and biotic interactions (Albert et al., 2012) and also understand the latitude diversity gradient (Lamanna et al., 2014). These traits have gained popularity in understanding effects and responses of species and communities to differences in climate (Ordoñez et al., 2009) and are being used extensively in climate change models (Verheijen et al., 2015).Plant functional traits are generally measured for leaves, stem, roots and seeds.

This study focuses on leaf functional traits. Leaf traits are the most commonly studied plant functional traits. Leaves are the primary photosynthetic organ and changes in leaf properties have a direct impact on the performance and fitness of the plant. Moreover, leaves are abundant implying a large investment by the plant. They have a high surface area in contact with the environment and any changes in the environment will be

reflected in the leaves. Additionally, it is comparatively easier to measure leaf traits than stem or root traits.

Examples of leaf traits are leaf mass per unit area (LMA), leaf lifespan (LL), leaf nitrogen content (LNC), photosynthetic efficiency (P_{net}), etc. Studies over the past decade have shown that leaf traits, not only show strong relation with environmental conditions but also show strong correlations with other leaf functional traits (Reich et al., 1999). A few traits are a part of the Leaf Economic Spectrum (LES) which helps in understanding the plant investment to returns dynamics (Wright et al., 2004). Production of leaves is seen as an investment made by the plant and the products of photosynthesis as the returns to the plant. Plants try to maximize the returns in their environment. Examples of such relationships are Leaf mass per unit area-Leaf lifespan, Leaf nitrogen content- Leaf phosphorous content, LMA-LNC, etc.

The vast majority of the work on leaf functional traits has focused on temperate plants and the tropics have received less attention. Studies from the dry tropics are particularly sparse, as is data from the Indian subcontinent. Of the eighteen studies that have examined leaf functional traits, most came from one research group that has done extensive work in dry deciduous forests of Central India (Chaturvedi et al., 2011a, 2012; Kushwaha et al., 2010; Lal et al., 2001). The others include one study each from the Himalayas (Singh et al., 2014), Gujarat (Mehta et al., 2012), Assam (Athokpam et al., 2013) and the Eastern Ghats (Stephen et al., 2012).

Research on plant functional traits have focused on interspecific variation and implicit in these studies is the assumption that variation within species is negligible. However, recent work has documented up to 55% variation within species at multiple levels: individuals, populations and communities (Albert et al., 2011). Intraspecific variation is defined as differences in trait values within and/or between individuals of a species. This can arise due to plasticity in the trait, genetic variation between individuals (Warren et al., 2006), or as a combined effect of both (Albert et al., 2011). Inclusion of intraspecific variation in community assembly models have improved the sensitivity and predictive power of models (Paine et al., 2011). Species with large intraspecific variation can adopt trait values permitted in a novel environment, and also allow co-existence of

species by niche partitioning (Jung et al., 2014)(Lichstein et al., 2007). Using transplant experiments to a novel environment, Warren and Lake (2012) show that species that can survive in novel environments conditions have higher intraspecific variation in functional traits. Similarly, species with large geographical distribution are reported to have higher within species variation in specific leaf area (Sides et al., 2014). The direction of change in trait values within species across environmental gradients is consistent with the direction of change in traits between species (Walters and Gerlach, 2013). This is important to understand the effect of climate change on species and predict the direction of range shifts. Within species variation in traits at the extremes of species' distribution limits determine their ability to disperse to a newer environment or persist in the face of changing environments (Nicotra et al., 2010; Thuiller et al., 2008; Valladares et al., 2014). Extensive work is required to understand the effect of intraspecific variation on population density, transient dynamics, etc. (Bolnick et al., 2011) and its effect on patterns at larger scales (Albert et al., 2011). Studies at the regional scale or across biogeographic ranges will help in correctly parametrizing within species variation in global models (Niinemets, 2015) and in planning assisted migration of species for conservation (McLean et al., 2014).

Majority of the understanding of traits and their variation across environments comes from controlled experimental work that has been carried out on a variety of species and life forms. Poorter et al. (2009) describe general patterns in trait variation across these studies. Leaf mass per unit area (LMA) which is the ratio of leaf dry mass to area and a measure of leaf level cost to light interception, increases with shortage of water and increased irradiance. Two other traits closely linked to LMA and carbon dynamics in the leaf are Leaf Dry Matter Content (LDMC) and Leaf Carbon Content, defined as the ratio of leaf dry mass to saturated fresh mass of the leaf and percentage of Carbon present in 100mg of leaf dry matter, respectively. While LDMC is a measure of leaf density (Garnier et al., 2013) and cellular metabolism (Domínguez et al., 2012), LCC is used to estimate structural components of the leaf (Albert et al., 2010). LDMC is similar to LMA in its response to water and light availability (Poorter et al., 2009) but not much is known for LCC. An important trait in the leaf economic spectrum (Wright et al., 2004) that is closely linked to plant photosynthesis is Leaf Nitrogen Content (LNC). It is percentage of

Nitrogen present in 100mg of leaf dry matter. Results for variation in LNC to environment changes has been equivocal. Leaf area (LA) is helpful in understanding plant-light and plant water relationship (Garnier et al., 2013) as it is linked to light interception and transpiration. Leaves exposed to high irradiance and/or low water are smaller in area (Poorter et al., 2009). While trait responses can be generalized from experimental studies, it might not hold true in field studies on natural populations.

Studies on intraspecific variation in leaf traits are limited for naturally occurring species. Of forty field studies that explicitly examine intraspecific variation in leaf traits, half have studied the effects of water availability and light intensity. The gradients in annual rainfall across which intraspecific variation in leaf traits have been investigated are in the range of 500-1500mm for temperate studies, extending to 3000mm in tropical studies (Appendix 1). These studies show that within species, Specific Leaf Area[1] (Inverse of leaf mass per unit area) increases (Baruch, 2011; Chaturvedi et al., 2011b; Gouveia and Freitas, 2008; McLean et al., 2014; Wang et al., 2014) and Leaf dry matter content decreases with increasing rainfall (Chaturvedi et al., 2011b; Kichenin et al., 2013). There are no clear patterns of Leaf nitrogen content[2] with rainfall (Gotsch et al., 2010; McLean et al., 2014), and it likely to be dependent on soil nitrogen content (Walters and Gerlach, 2013). Contrasting results were described for leaf Carbon Content (LCC) with Gotsch et al. (2010) reporting higher LCC for a wetter site and Méndez-Alonzo et al. (2008) reporting higher LCC in a drier site. No field studies have examined intra-specific variation in leaf area.

Variation due to light has been studied at the level of individuals (present in open vs. closed canopy forests) and within individuals as a function of position of leaves in the canopy (sun vs. shade leaves). While inter-individual differences are important in community assembly models, studying plasticity due to light between leaves present in different strata within a tree helps estimate the range of plastic responses that

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[1] Specific leaf area (SLA) is leaf area per unit mass and is the inverse of leaf mass per unit area (LMA). In this document these two traits have been used interchangeably depending on the context of the study being cited. While the larger conclusion from both will the same, LMA cannot be obtained just by taking the inverse of SLA.
[2] In this document, Leaf Nitrogen Content, Leaf Carbon content, Leaf Phosphorous content and net photosynthesis are all on a mass basis unless explicitly mentioned.

individuals can exhibit, and this is likely to be related to species distribution (Gratani et al., 2006). LMA shows high plasticity as a function of changes in the light (140% higher SLA for shade leaves than for sun leaves) (Poorter et al., 2006). Leaf area has been reported to increase by as much as 30% in shade leaves (sack), and LCC exhibits marginal increases (4% for sun leaves) (Poorter et al., 2006). No consistent differences have been reported for LDMC (Weerasinghe et al., 2014) and LNC (Mitchell et al., 1999; Weerasinghe et al., 2014; Wyka et al., 2012) as a function of light.

Few studies have examined intraspecific variation in traits for multiple species across large biogeographic scales and associated environmental gradients. The modal number of species in these studies is between1 -20.These studies were either single species studies over multiple sites (Niinemets, 2015; Ogaya and Peñuelas, 2007) or multi-species study at a single site (Kang et al., 2013; Messier et al., 2010). A multi-species and multisite study by Long et al. (2011) spans a short geographical range. Most of the multi-site studies address questions on intraspecific variation with sites which are a few tens of kilometers apart. Only eight previous studies which have examined intraspecific variation across sites that are at least 500 km apart. Of these, six studies (Baruch, 2011; Dawson and Bliss, 1989; Fajardo and Piper, 2011; McLean et al., 2014; Méndez-Alonzo et al., 2008; Niinemets, 2015) are all for a single species spread over multiple sites. With the exception of Dawson and Bliss (1989) who compare montane and artic populations more than 3000km apart, the comparisons are between sites in the range of 500-1000km. Only two multispecies studies, Richardson et al (2013) which studies 4 species spread over 700km and Wang et al. (2014) address the effect of rainfall differences between two sites 950km apart on 92 species. Of these, Baruch, (2011) and Méndez-Alonzo et al. (2008) are the only tropical studies. Tropical and the dry tropical forests are under-represented in large biogeographic studies and it is especially true with respect to India. Till date, only one study from central India has addressed questions related to intraspecific variation (Chaturvedi et al., 2013).

Relationship between traits are often studied as they suggest possible trade-offs or constraints and that only certain combinations of traits and hence, strategies can be expressed. Alternately, different combinations of traits can be expressed but only

certain ones are selected for and are manifest under specific environmental conditions. For example, there is a strong negative correlation between leaf mass per unit area and leaf nitrogen content (Wright et al., 2004). This relationship defines a spectrum of leaf strategy ranging from short lived-high photosynthetic efficiency leaves (LMA-high LNC) to long lived-low photosynthetic efficiency leaves.

The relationship between traits can vary between sites by having a different slope, different intercept or by shifting along the slope (Warton et al., 2006; Wright et al., 2001). If the slope of the relationship LMA-LNC changes between environments, it means that photosynthetic efficiency drops to different degrees with increase in leaf biomass in different communities. However, if only the intercepts change between different environments, it suggests that different environments have different nitrogen content and therefore different photosynthetic efficiency for the same construction cost (LMA). For a given nitrogen content, the drier sites have higher LMA, meaning that a plant makes higher investment in drier sites to achieve similar photosynthesis. Conversely, at a given LMA, drier sites have higher nitrogen content in the leaf. This is beneficial as it is expected to improve water use efficiency at the dry sites (Wei et al., 2011) but it incurs a higher construction cost. The shift in trait relationships is thought to result from changing species composition at different sites (Wright et al., 2001). But such a shift can result from intraspecific variation in traits. It is important to understand how intra-specific variation in traits influence relationships between traits. This will help us understand the underlying constraints at the physiological level that can affect patterns at a larger scale. Alternately, this may shed light on how specific environmental conditions may select for specific combinations of traits. A study from the Tibetan plateau on shrubs, reported higher leaf nitrogen per unit mass at a drier site for a given Specific Leaf Area. Wright and Sutton-Grier (2012) tested for the existence of LES relationships in controlled pot experiments for effect of water and nitrogen availability and found that SLA-LNC was congruent with the global trend but the slopes for the water availability experiment did not differ across the treatments. However, the effect of treatments was seen as a shift in intercept and along the slope. Similar trends were observed in a tropical field study where each of the species showed an increase in LNC for a given SLA from wet to dry site (Gotsch et al., 2010). For comparison between light

levels, it is expected that leaves at higher irradiance is have higher nitrogen content for a fixed leaf mass per unit area (Wright et al., 2001).

This study investigates patterns in intraspecific variation at large biogeographic scales with contrasting rainfall patterns in the Indian tropical dry forests. The sites were chosen to provide contrasts in total annual rainfall and duration of the dry season. Nagarjunasagar-Srisailam Tiger Reserve has very low rainfall and high seasonality (extensive dry period) and the Southern Western Ghats has very high rainfall and low seasonality (short dry period). Northern Western Ghats is intermediate with high rainfall but high seasonality.

The primary objectives of this study were to examine intraspecific variation in leaf functional traits in tropical tree species. This study examines two different components of intraspecific variation: a) Geographic variation between individuals across large biogeographical and associated environmental gradients. More specifically it investigates whether the trait values differ due to difference in magnitude or seasonality in rainfall. b) Plasticity within individuals as a function of the canopy light position of leaves (sun vs. shade). In addition, does this plasticity in trait vary at different sites? It further explores the relation between plasticity within an individual and variation between biogeographic regions to check whether higher plasticity is associated with higher intraspecific variation between sites. Lastly, it examines how the relationship between Leaf mass per unit area and leaf nitrogen content varies across environmental conditions and compares it with the existing interspecific trends.

Methodology:

Site description: The three sites in this study were primarily chosen to provide a range of mean annual rainfall and duration of dry period. They are situated in three different biogeographic regions in the peninsular India and also differ in altitude, soil properties, and forest type (Table 1). **Nagarjunasagar Srisailam Tiger Reserve (NSTR)**, Andhra and Telangana (16.3000° N, 78.9833° E) is situated in the Eastern Ghats and is a very dry site which receives less than 800mm of rainfall spread over four months. The extended dry duration is 8 months long. The forest is categorized as tropical dry deciduous (Champion and Seth) though there are areas with moist deciduous forest patches. The dominant tree species in the region are *Anogeisus latifolia, Chloroxylon sweitenia, Terminalia elliptica, Grewia orbiculata* and *Phyllanthus emblica*.

Bhimashankar Wildlife Sanctuary (NWG), Maharashtra (19.1320° N, 73.5540° E) in the Northern Western Ghats has high total annual rainfall but all of this precipitation is received in just five months during the monsoons and the site remains dry for the next seven months. The forest type can be classified as tropical moist deciduous (Champion and Seth). However, there are three distinct microhabitats, the open and dry crest forests, partially closed canopy moist forests along the slopes and a wet and closed canopy valley forests. *Memeceylon umbellatum* is the most abundant species in the region followed by *Catenugarum spinosa, Bridelia retusa, Mangifera indica* and *Syzygium cumini*. **Kulathupuzha Reserve Forests (SWG)**, Kerala (8.9082° N, 77.0555° E) in the Southern Western Ghats receives high rainfall from the south west and north east monsoons spread over eight months. The dry period here is less than four months. The forests are semi-evergreen (Champion and Seth) in nature, and the dominant species are *Helicteres isora, Aporosa cardiosprema, Xanthophyllum arnottianum, Ixora brachiata* and *Strombosia zeylanica*.

Species selection: The species were selected to maximize overlap between the three sites. From the available species list for the three regions, 32 species were identified that were present in two or more sites. Twenty nine of these 32 species were collected, but sufficient number of individuals of three other potentially overlapping species could not be located. Twelve species were collected from all three sites, and the rest were present in pairs of sites. A total of 22 species were collected in NSTR, 26 in NWG and

22 in SWG, which add up to a total of 70 species X site combinations (Appendix 2). Overall, 3460 leaves were collected from 346 individuals across sites and species. Only in NWG, most of the dominant species were sampled. In NSTR, *Terminalia elliptica* was collected along with other common and occasionally occurring species. Most of the species collected in SWG are occasional or rare.

Table 1: Description of sites. Site characteristics for Nagarjunasagar-Srisailam Tiger Reserve (NSTR), Northern Western Ghats (NWG) and Southern Western Ghats (SWG). The Carbon and Nitrogen values for the soil were quantified during this study. MAP is mean annual precipitation and MAT is mean annual temperature. If less than 100mm/month of rainfall is recorded it is defined as a dry month. The climate data were extracted from a high resolution global dataset of 30 year climatological normals for the period between1960-1990 (New et al., 2002).

	NSTR	NWG	SWG
Coordinates	16°18'N, 78°58'E	19°07'N, 73°33'E	8°54' N, 77°03'E
Altitude(m)	~100	~1000	~600
MAP(mm)	745	2266	1945
MAT(°C)	26	23	26
Duration of dry period	8	7	4
Forest type	Dry deciduous	Moist Deciduous	Semi-evergreen
Soil C (%)	1.620	4.190	1.950
Soil N (%)	0.169	0.303	0.195

Measurement of leaf traits: All the samples were collected between 20th of July and 10th of November 2014. Only recently matured leaves that had flushed after the onset of the monsoon, in the presence of sufficient water, were collected. Samples from NSTR, which has the shortest dry season, was collected from 20th July to 9th August, 2014; followed by collection in the NWG from 30th August to 27th September, 2014; and, lastly samples from the SWG from 4th October to 10th November, 2014. For each species, five randomly picked adult individuals at each site were sampled. From each individual, leaves were collected from branches at two different strata of the canopy- sun exposed

outer canopy and shaded inner canopy. Effort was taken to obtain the sun exposed leaves from top of the canopy and the leaves in the shade from under the centre of the canopy, whenever possible. The first fully expanded mature leaves in each twig was collected for further processing. The leaves were collected in labelled paper bags which were stored in moist Ziploc bags until the samples were brought to the laboratory.

All methods used for measuring the leaf traits are adopted from (Garnier et al., 2013). Once the samples were brought back to the laboratory, five leaves each from sun and shade strata for every individual was stored in the Ziploc bags filled with sufficient water and saturated at 4°C for 12 hours. Post saturation, the leaves were dabbed with clean tissue to remove any water from the surface, weighed, scanned with Canon 600 LIDE at 300dpi, and dried at 60°C for 72 hours. At the end of 72 hours the leaves were weighed and the dry weight was noted. A thin layer of white adhesive or nail polish was used to obtain impressions of stomata from fresh leaves. Slides of these peels were prepared using transparent sticky tape. The peels were made for a single leaf in each of the two light strata of an individual. The slides were stored for further analysis. Extra leaves collected were also dried at 60°C for 72 hours for further processing to obtain Carbon, Nitrogen and Phosphorus contents.

Leaf area was calculated using an image analysis software ImageJ (Schneider et al., 2012). LDMC and LMA were calculated as ratios of dry weight and saturated fresh weight and dry weight and leaf area, respectively for each leaf. The dried leaves from each strata of an individual were pooled together, ground and sieved at 40 microns for obtaining LCC, LNC and LPC.

LCC and LNC: The nutrient concentrations were measured using the Leco CN microanalyser where ~0.1000g of organic material is first completely oxidized. The CO_2 thus formed is analysed in an infrared cell which measures the absorption spectra. All the NO_x gases formed during combustion are reduced using copper fillings and then analysed using a thermo-conductivity cell. The instrument provides values for concentration as a mass percent (mg/g).

LPC: For estimation of Phosphorus, the powdered leaf sample had to be digested. During the pre-digestion step 0.15g of dry leaf matter was treated with 5ml of 70%

HNO$_3$ in a capped Teflon tube and left overnight. This was followed by an incubation step of two rounds- 75°C for 10min and 109°C for 15 min. Once, the samples cooled, 1ml of 30% H$_2$O$_2$ was added to the tube and incubated at 109°C for another 15 minutes. On cooling the solution was filtered and diluted to 20 ml with distilled water and stored at 4°C. This solution was analysed using the ICP to obtain concentrations of P in mg/L. This is a standardized procedure adapted from (Huang et al., 2004).

The leaf peels were imaged with a DIC microscope. Images were taken along a randomly chosen line at three points from the midrib. The slides were imaged at both 10X and 40X to obtain stomatal density and stomatal size.

Both LPC and stomatal traits are yet to be processed and analysed and will not be included as a part of this thesis.

Soil Analysis: Soil cores were collected from each of the three sites to compare their soil Carbon, Nitrogen and Phosphorous. Five cores each of 15 cm deep was collected from every microhabitat. There were 2 different microhabitats in NSTR and both Bhimashankar and Kulathupuzha had five different microhabitats. The microhabitats were selected based on where the trees had been sampled. These soil samples were collected in Ziploc bags and dried in the lab at 60°C for 96 hours. They were finely ground using a mortar and pestle and sieved and analysed in the Leco CN microanalyser for Carbon and Nitrogen content. The procedure was similar to measuring leaf nutrients except for the longer combustion step. For Phosphorus content, the soil was digested with 5 ml of 70% HNO$_3$ overnight and incubated at 175°C for 10min. On cooling it was diluted to 20 ml and stored at 4°C to be analysed by ICP.

Data analysis: All the analysis has been carried out in R 3.1.3 (R Core Team, 2015). The packages that have been used are 'dplyr' (Wickham and Francois, 2015) and 'ggplot2' (Wickham, 2009) for data managing and plotting, respectively. The package 'smatr'(Warton et al., 2012) was used to fit standardized major axes.

Effect of site and species on the functional traits: A two way ANOVA with site, species and their interaction term was performed. Data for all the traits except LCC were log transformed to meet the condition of normality. LCC data was square transformed. In

cases where data could not be transformed to normality, Kruskal-Wallis non-parametric test was performed to check for statistical significance.

Pairwise site effects: In the whole data set, sites differ in the number and type of species collected. To confirm the intraspecific differences between the sites balanced ANOVA's were performed for three subsets of data: a) Species present at all three sites (n=12 X 3); b) Species present only in NSTR and NWG (n=18 X 2); and, c) Species present only in NWG and SWG (n=19 X 2).

Effect of site and species on plasticity due to light: A plasticity index was calculated as the difference between the means of sun and shade leaves of an individual normalized by the species means of sun leaves. The plasticity index was used as a response variable in a two way ANOVA with site, species and their interaction term. When data was non normal and could not be transformed to normality, non-parametric Kruskal-Wallis test was used to assess significance. Plasticity is used synonymously with plasticity due to light, unless mentioned otherwise.

Relationship between traits: A Standardized Major Axis (SMA) was fit for pairs of traits to test whether their slopes and intercepts were different.

Results

Variation in traits between species and sites

As expected there was significant variation between species in all the leaf traits examined (Table 2a). Traits were also significantly different across sites. The interaction effect of site and species though significant was much smaller than the main effects (Table 2, Appendix 3). As all species were not present in all sites, a balanced ANOVA was performed for the 12 species that are present at all three sites. The results for this was congruent with the previous analysis except for the effect of site on LNC (Table 2b, Appendix 4).

Table 2: Effect of species and site on traits. Summary table with values of significance (P values) from a two-way ANOVA for effect of species and site on the five different traits for two subsets of data: a) the whole data has 29 species and 70 species-site combinations over three sites. b) The balanced data has 12 species present in all three sites, 36 species-site combinations. The traits are leaf mass per unit area (LMA), leaf dry matter content (LDMC), leaf area (LA), leaf carbon content (LCC) and leaf nitrogen content (LNC).

	LMA	LDMC	LA	LCC	LNC
a) Whole data					
Species	<0.001	<0.001	<0.001	<0.001	<0.001
Site	<0.001	<0.001	<0.001	<0.001	0.0179
Site X Species	<0.001	<0.001	<0.001	<0.001	<0.001
b) Balanced data					
Species	<0.001	<0.001	<0.001	<0.001	<0.001
Site	<0.001	<0.001	<0.001	<0.001	**NS**
Site X Species	<0.001	<0.001	<0.001	<0.001	<0.001

Leaf mass per unit area was lower in the wet site, Southern Western Ghats, compared to the dry Nagarjunasagar-Srisailam Tiger Reserve and seasonally dry Northern Western Ghats (Fig 1a). LMA in NWG was marginally higher than in NSTR (p=0.048, post-hoc analysis). In the species examined, *Memeceylon umbellatum,* had the highest LMA values and also shows the largest change in LMA across sites being 70% lower in the SWG than in the other two sites. However, species like *Carallia brachiata, Catenugaraum spinosa* and *Mallotus philippensis*, exhibit little or no difference in LMA across sites.

Similarly, Leaf Dry Matter Content (LDMC), was lower in the SWG (Fig 1b), but there was no difference between NSTR and NWG. Again, *M. umbellatum* had the largest change in LDMC, being ~30% lower in SWG compared to the seasonally dry NWG. Unlike LMA, the direction of change in LDMC was inconsistent across species. Species like *C. brachiata, Terminalia elliptica* and *Tectona grandis* did not differ significantly between the sites.

Species identities explained a relatively larger proportion of the variation in leaf area (LA) (Appendix 3c). However, when the large leaved species (*Leea indica, T.grandis* and *Cassia fistula*) are excluded from the analysis, the effect of species and site becomes comparable. There is a general trend of higher leaf area for individuals at the wetter site, SWG, compared to the other two sites.

For LNC, while both species and site had significant effects in the overall model, the effect of site was small, and this effect was not significant in the balanced model. This implies that the small but significant effect in the overall model may have been a consequence of differences in the species in the sites, and that variation in leaf Nitrogen content can be primarily be attributed to differences between species (Appendix 3e, Fig1E). *Albizia lebbeck*, which is a nitrogen fixing plant, has the highest LNC among the species considered followed by C. fistula.

Leaf Carbon content showed idiosyncratic effects of species and site (Appendix 3d, Fig 1d). The wet site had higher LCC compared to both NSTR and NWG, which are dry and seasonally dry sites, respectively. NSTR and NWG were not different from each other. *Bridelia retusa* and *Olea dioica* had the highest carbon content and *Morinda pubescens,* had the least carbon content.
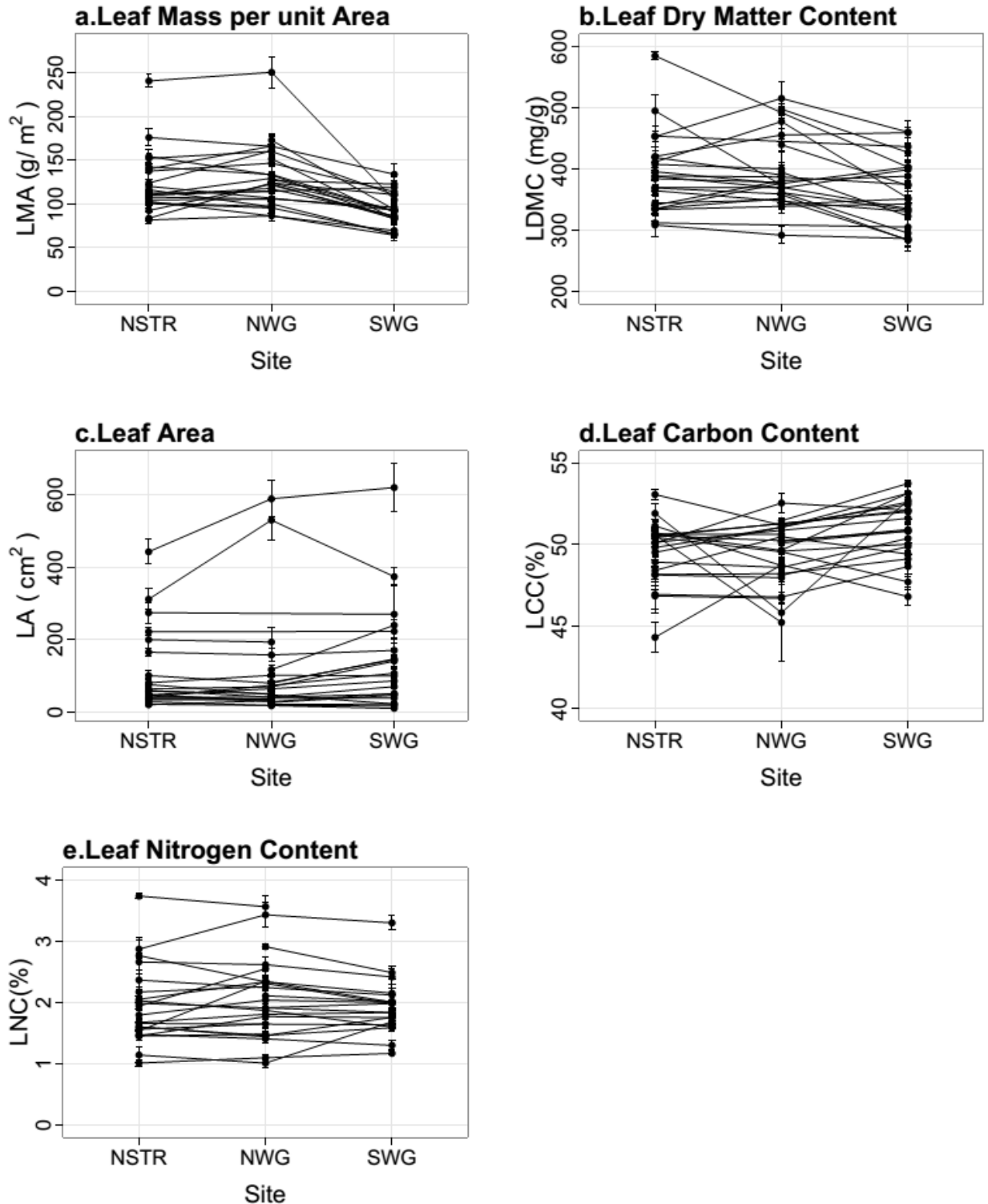
Fig 1: Trait variation across the three sites. Species are dots. Traits are a) Leaf mass per unit area b) Leaf dry matter content c) Leaf area d) Leaf Carbon content e) Leaf Nitrogen Content. The x-axes are the sites, Nagarjunasagar-Srisailam Tiger Reserve, Northern Western Ghats and Southern Western Ghats, arranged in increasing order of water availability.

Comparison between sites to evaluate effect of seasonality and total rainfall

Pairs of sites were compared to understand the importance of total annual rainfall (NSTR vs NWG) and number of dry months (seasonality in water availability) (NWG vs SWG) in determining the trait variability. Interspecific variation was significant for the all traits and the interactive effect of species and site is much smaller compared to the main effects. For both the comparisons, variation in leaf nitrogen content was predominantly explained by interspecific differences, with a smaller effect or no effect of site (Appendix 5e and 6e).

For the rest of the traits examined, the comparison between NSTR and NWG revealed no significant differences between the sites except for a marginal difference in Leaf Carbon Content (Table 3a). In contrast, the comparison between NWG and SWG shows significant differences for LMA, LDMC and LCC (Appendix 6a, 6b and 6d). Though leaf area is different across sites, most of the variation is explained by difference between species. The trends in variation of traits is as described earlier.

Table 3: Comparison between sites that differed in total rainfall or seasonality of rainfall.
Summary table with values of significance (P values) from a two-way ANOVA for effect of species and site on the five different traits for pairs of sites to compare: a) Magnitude of rainfall - Nagarjunasagar-Srisailam Tiger Reserve vs. Northern Western Ghats for 18 species; and, b) Seasonality of rainfall - Northern Western Ghats vs. Southern Western Ghats for 19 species. The traits are leaf mass per unit area (LMA), leaf dry matter content (LDMC), leaf area (LA), leaf carbon content (LCC) and leaf nitrogen content (LNC).

	LMA	LDMC	LA	LCC	LNC
a) NSTR-NWG					
Species	<0.001	<0.001	<0.001	<0.001	<0.001
Site	**NS**	**NS**	**NS**	0.0436	<0.001
Site X Species	0.0031	<0.001	<0.001	<0.001	<0.001
b) NWG-SWG					
Species	<0.001	<0.001	<0.001	<0.001	<0.001
Site	<0.001	<0.001	<0.001	<0.001	**NS**
Site X Species	<0.001	<0.001	<0.001	<0.001	<0.001

Variation in traits between sun and shade leaves

Plasticity due to light has been defined as the difference between the trait values of sun and shade leaves, normalized by the average trait value of the sun leaves of the species at that site. In general, all the five traits displayed plasticity to light but they differed in the magnitude of plasticity. Also, species differed in their plasticity with ranges extending from no change to almost 50% increase in the trait value. Leaf area was the most plastic trait with an average plasticity of 18% across all species. LMA was the second most plastic trait with 15% plasticity followed by LDMC and LNC at 6% and 3% plasticity, respectively. The least plastic trait was LCC which was just 1% different between sun and shade leaves.

Sun leaves which are exposed to harsher environmental conditions, had higher LMA than the shade leaves. Plasticity due to light for LMA ranges from 0.6% in *Atlantia racemosa* to almost 27% in *Terminalia chebula*. LDMC too was higher for the sun leaves and the trend was consistent across species with the exception of a few species. Change in Leaf dry matter content ranged from 2% in *Olea dioica* to 11% in *Mitragyna parvifolia*. Leaf Carbon content was the least plastic trait ranging from 0-4%, and the sun leaves had higher carbon content. Few species had higher carbon in the shade leaves. The light limited shade leaves were much larger than the sun exposed leaves. While a majority of the species show this trend, a few them are either not different between sun and shade or have larger area for sun leaves. Plasticity in leaf area ranged from almost no plasticity in *Tectona grandis* to 43% difference in *O. dioica*. Though leaf Nitrogen content is plastic to light, ranging from 0 to 8%, there is no consistent trend with some species having higher LNC for shade leaves and others for the sun leaves.

Variation in plasticity due to light between species and sites

Species differed in their plasticity due to light for all traits except for leaf mass per unit area and leaf dry matter content (Table 4). Site explained most of the variation in plasticity due to light for LMA and LCC (Appendix 8a and 8d). The effect of interaction between site and species on plasticity due to light is low compared to the main effects. The results from the balanced analysis qualitatively agreed with the above results (Appendix 6, Table 4).

Table 4: Effect of species and site on plasticity due to light. Summary table with values of significance (P values) from a two-way ANOVA for effect of species and site on plasticity due to light for the five different traits for two subsets of data. Plasticity due to light is the difference between sun and shade trait values normalized by the average sun leaf trait values for a species at a site (Δ Trait). a) The whole data has 29 species and 70 species-site combinations over three sites. b) The balanced data has 12 species present in all three sites, 36 species-site combinations. The traits are leaf mass per unit area (LMA), leaf dry matter content (LDMC), leaf area (LA), leaf carbon content (LCC) and leaf nitrogen content (LNC).

	ΔLMA	ΔLDMC	ΔLA	ΔLCC	ΔLNC
a) Whole data					
Species	**NS**	**NS**	<0.001	<0.001	<0.001
Site	<0.001	0.0354	0.0475	<0.001	0.0109
Site X Species	0.0114	0.0003	0.0063	<0.001	0.0050
b) Balanced data					
Species	**NS**	**NS**	<0.001	0.0055	<0.001
Site	<0.001	0.0188	0.0061	<0.001	**NS**
Site X Species	**NS**	0.0010	**NS**	<0.001	0.0256

Plasticity due to light in LMA differed across sites and the general trend is a marked reduction in plasticity at the wetter Southern Western Ghats (Fig 2a). Barring a few species, plasticity in LMA at NSTR and NWG was either the same or for some species higher in the latter. Comparing across all the species and site combinations, it was noted that *M.umbellatum* displayed the highest plasticity at NWG. Also, it was the species which had the least plasticity due to light in SWG. For LCC, variation in plasticity was the largest (-6 to 5%) for NSTR. Trees at SWG and NWG were mostly not plastic (Fig 2d). LDMC too differed between sites but, there are no consistent trends between the sites due to a comparable interaction effect of species and site (Appendix 8b). Variation in leaf area plasticity was dependent both on the species and site effects independently (Table 4a). However, there were no general trends in plasticity across the sites. Though interaction was insignificant, plasticity in species like *M. phillipensis* and *Mangifera indica* differed between sites, ranging from 10% in NWG to 60% in NSTR.
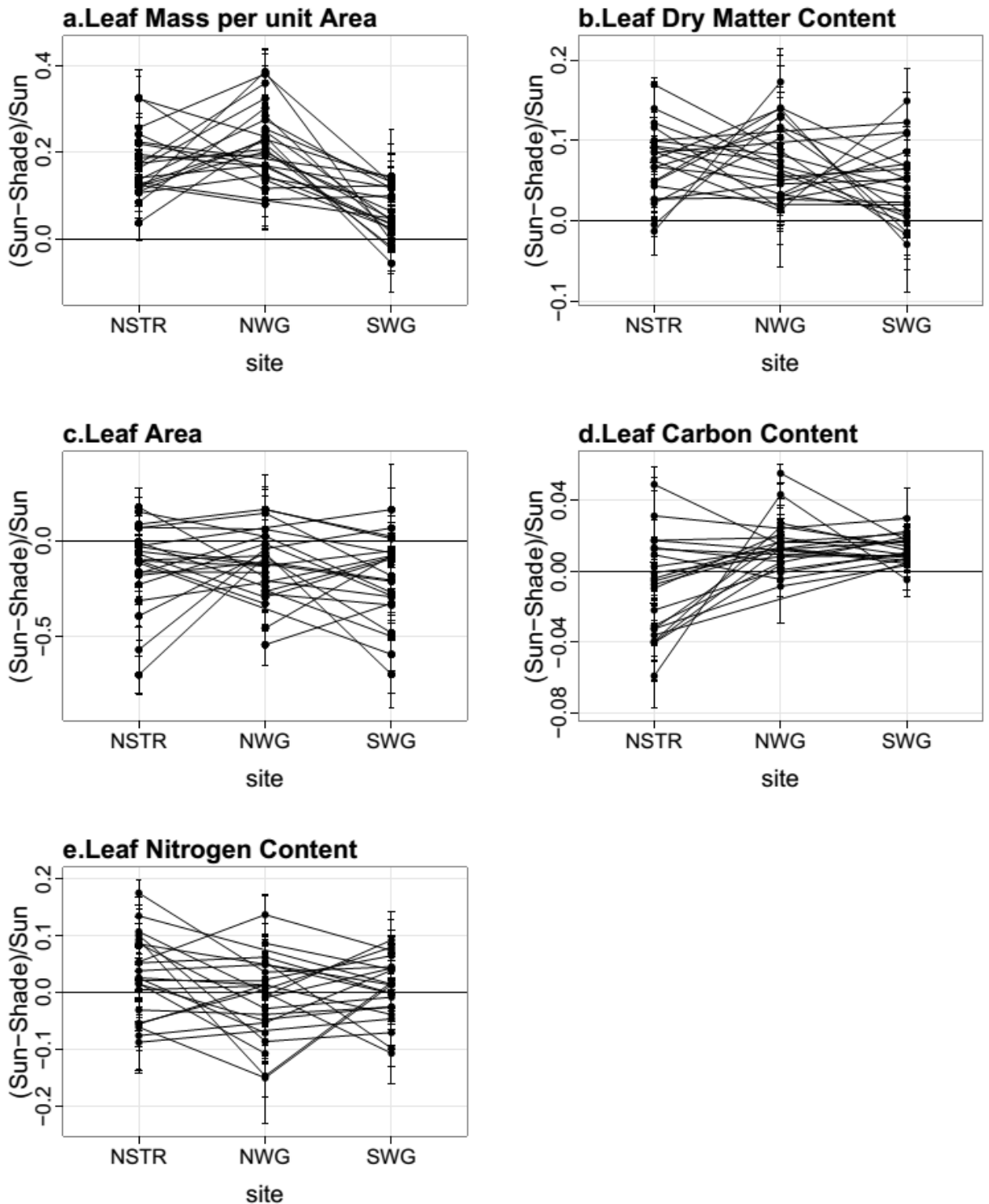
Fig 2: Plasticity due to light for traits across the three sites. Species are dots. Traits are a) Leaf mass per unit area b) Leaf dry matter content c) Leaf area d) Leaf Carbon content e) Leaf Nitrogen Content. The x-axes are the sites, Nagarjunasagar-Srisailam Tiger Reserve, Northern Western Ghats and Southern Western Ghats, arranged in increasing order of water availability and seasonality.

The effects of site and species are comparable for plasticity of leaf nitrogen content and no consistent trend was noticed across sites (Fig 2e). The highest plasticity was shown by *Terminalia elliptica* at NSTR with 17.5% difference between the light levels and the least by *Bridelia retusa* at SWG with 0.1% difference between sun and shade.

Relationship between intra-specific variation due to light environment and sites

Different traits had different levels of intra-individual variation (plasticity due to light) and inter-site variation, with the latter being much greater than the former. In general, the order of variation in traits for both within individual variation and across site variation was the same, with leaf area being the most variable trait and LCC being the least variable (Table 5). Maximum variation in leaf area is reported for *C. spinosa* at almost 111.1% difference between the sites. *M. umbellatum* is the most variable for both LMA and LDMC. *S. cumini* and *T. grandis* varied the most across sites for LNC and LCC, respectively. Species with the maximum amount of plasticity due to light were not the same as the ones that had high inter-site variation. *Terminalia chebula*, was the most plastic to light in LMA at 26% difference between sun and shade leaves. For LDMC, 11% difference was the maximum plasticity recorded for *Mitragyna parvifolia. Olea dioica* was almost one and a half times larger in area for shade leaves than the sun leaves. The plasticity in LNC and LCC was much less compared to the other traits with maximum plasticity being 8.8% (*Cassia fistula*) and 3.6% (*Alstonia scholaris*), respectively. Only for LDMC, the same species, O.dioica, was the least variable both across sites and between sun and shade leaves. However, there was no significant relation between inter-site variation and plasticity due to light for the five traits considered.

Table 5: Variation in traits across sites and trait plasticity due to light for species. a) Variation in traits across sites for different traits and species, measured as the difference between the maximum and minimum of the mean trait values across three sites for a species normalized by the average trait value for that species. Mean value is the average over all the species. b) Plasticity due to light measured as the difference between sun and shade trait values normalized by the average sun leaf trait values for a species at a site. Mean is the average plasticity of a species over all three sites. The maximum and minimum columns report the highest and lowest values of inter-site variation and intra-individual variation, respectively. The traits are leaf mass per unit area (LMA), leaf dry matter content (LDMC), leaf area (LA), leaf Carbon content (LCC) and leaf Nitrogen content (LNC).

a) Trait	Maximum Variation %	Species	Minimum Variation%	Species	Mean Variation %
LMA	73.3	Mu	0.6	CasG	29.3
LDMC	36.7	Mu	1.5	Ol	11.6
LA	111.1	CS	0.5	SchO	38.6
LCC	13.7	TecG	0.0	FI	4.0
LNC	52.6	SC	1.5	SchO	15.4
b) Plasticity					
ΔLMA	26.6	TerC	0.6	AR	15.4
ΔLDMC	11.5	Mpar	2.2	Ol	6.2
ΔLA	43.4	Ol	0.4	TecG	17.8
ΔLCC	3.6	Ash	0.0	Cfist	1.1
ΔLNC	8.8	Cfist	0.0	Carb	3.5

Note:The species mentioned in the table are *Memeceylon umbellatum* (Mu), *Cassine glauca* (CasG), *Olea dioica* (Ol), *Catunaragam spinosa* (CS), *Schleichera oleosa* (SchO), *Tectona grandis* (TecG), *Flacourtia indica* (FI), *Sygyzium cumini* (SC), *Terminalia chebula* (TerC), *Atlantia racemosa* (AR), *Mitragyna parvifolia* (Mpar), *Cassia fistula* (Cfist) and *Careya arborea* (Carb).

Variation in relationship between traits

The only consistent relationship across all site and light conditions was between leaf mass per unit area and leaf nitrogen content. The relationship was consistent for all the site and light conditions but did not differ from each other in their slopes. However, for a given value of leaf mass per unit area the dry NSTR and seasonally dry Northern Western Ghats had significantly higher leaf nitrogen content than the Southern Western

Ghats and conversely for a fixed leaf nitrogen content both the dry and seasonally dry sites had higher LMA. Though not significant, NWG had higher nitrogen values than NSTR for a given LMA (Table 6). Also, for all sites sun leaves had higher nitrogen content than the shade leaves at a given leaf mass per unit area except in the Southern Western Ghats. In the wetter site, there was no significant difference between the intercepts for the sun and the shade leaves, meaning that for a given value of nitrogen content, leaves from the two different light levels had the same LMA (Table 6).

Table 6: Summary of SMA slopes and intercepts. The slope and intercept values for Standardized Major Axis analyses for log (LNC) on x axis and log (LMA) on y axis, for each of the three sites and two different light conditions. The slopes are all significantly different from zero. Significant differences for different light effects is shown in bold font and difference across sites is shown by alphabets a-c. [Nagarjunasagar-Srisailam Tiger Reserve (NSTR), Northern Western Ghats (NWG), Southern Western Ghats (SWG), Sun refers to sun exposed leaves and Shade is the leaves collected from the canopy shade region]

Site	Light	Slope	Intercept
Whole data			
NSTR	Sun	-0.81	5.28[a]
	Shade	-0.88	5.11
NWG	Sun	-0.73	5.32[a]
	Shade	-0.79	5.14
SWG	Sun	-0.95	5.12[b]
	Shade	-1.17	5.18
Balanced data			
NSTR	Sun	-0.97	5.30[a]
	Shade	-1.05	5.19
NWG	Sun	-0.78	5.36[a]
	Shade	-0.86	5.18
SWG	Sun	-.0.80	5.03[b]
	Shade	-1.00	5.08
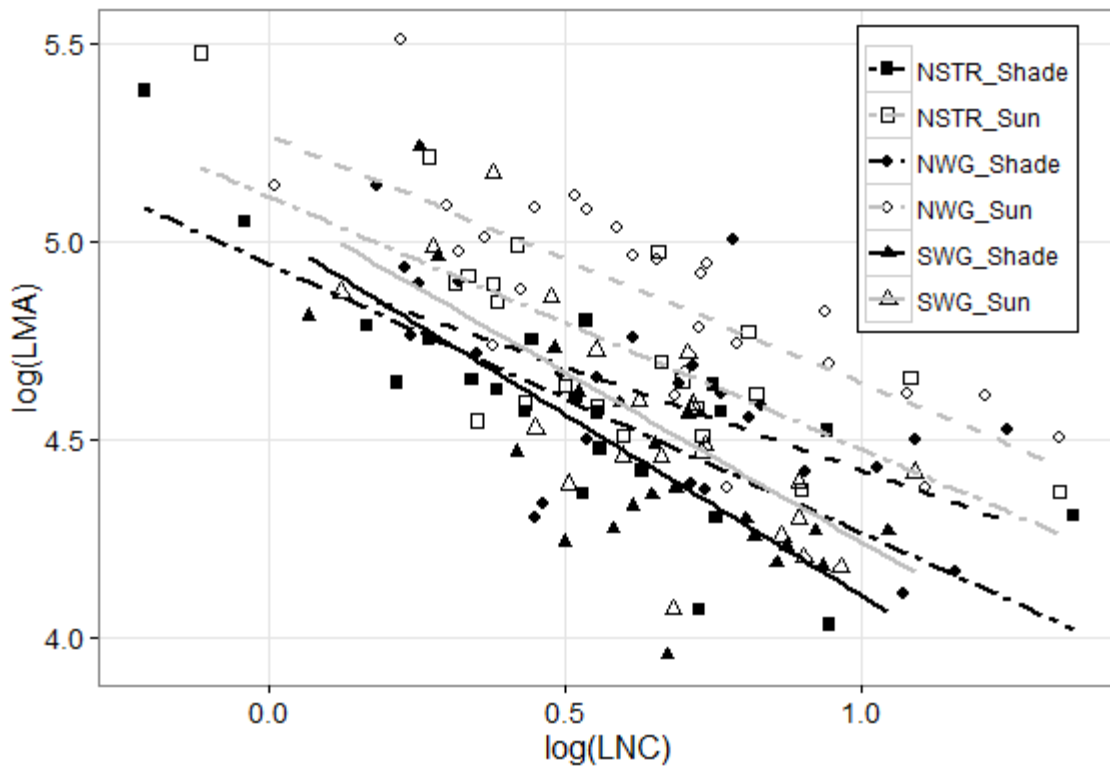
Fig 3: Standardized Major axis relationship between LMA and LNC. The relationship between log transformed leaf mass per unit area and leaf nitrogen content. Each panel represents one of the three different sites- Nagarjunasagar-Srisailam Tiger Reserve, Northern Western Ghats and Southern Western Ghats. At each site, the trait relationship is drawn for sun and shade leaves.

Discussion

This study examines intraspecific variation in leaf traits in 29 tropical forest tree species from three contrasting sites with large differences in rainfall amount and seasonality. There was considerable intraspecific variation in leaf traits, both within individuals between sun and shade leaves, and between sites. The results suggest that seasonality of water availability rather that cumulative rainfall through the year is very important in determining how leaf traits change. Additionally, plasticity in sun and shade leaves was dependent on water availability. Contrary to expectations there was no relationship between extent of variation in sun and shade leaves with the extent of variation seen across sites. Finally, in examining relationships between traits, as expected, there was a significant negative relationship between leaf mass per unit area and leaf nitrogen content. The slopes for this relationship did not differ but intercepts differed significantly for the sites and between sun and shade leaves. This suggests that there could be an underlying mechanistic constraint that limits the possible combination of trait values.

Variation between species: As expected species differed in their functional traits. The ranges of trait variation differed considerably for the five traits. Leaf area varied the most with variation up to 150 times while Leaf Carbon Content varied by less than 1.5 times, across species. Ranges of traits in study are quite large and comparable to the ones reported in global data. Comparing with values for tropical species from a global data set, GLOPNET (Wright et al., 2004), it was seen that ranges of both LMA and LNC in this study spans 60% or more of the recorded variation (Appendix 9). Interspecific variation in Leaf carbon content was greater than 60% of the variation (2.5-97.5 percent quantile variation) across all biomes seen for the trait (Kattge et al., 2011). Range of leaf dry matter content in this study was as large as 250-580 mg/g. Leaf area varied by two orders of magnitude from 9-1290 cm^2.

Variation across sites: All traits except for leaf nitrogen content varied across the three sites. However, most of the variation in leaf area and considerable amount of variation in both LDMC and LCC was due variation between species. For LMA, the differences between sites primarily explained the variation in LMA. For all the traits, the

interaction term between site and species, though significant, contributed very little to the overall variation.

Further comparisons on subsets of species was performed to ask how total amount of rainfall (NSTR vs NWG) and seasonality in rainfall (NWG vs SWG) affect the trait values. It was surprising to find that there was no difference for LMA, LDMC and LA between the dry NSTR and seasonally dry NWG even though the sites differed by a huge 2100mm of annual rainfall. Even though LCC differed across sites marginally, interspecific differences better explained the variation. The higher soil nitrogen in NWG explained the higher leaf nitrogen (Wright and Sutton-Grier, 2012) in NWG compared to NSTR. In contrast to these results, the comparison between the seasonally dry NWG and aseasonal SWG, all traits were significantly different except LNC. Site explained most of the variation in LMA. For LDMC, a comparatively large percent of variation was across sites but that of leaf area was mostly due to differences between species. As expected, the wet SWG had lower values for LMA and LDMC and higher leaf areas. Availability of water allows for cell expansion leading to less dense and elongated cells and therefore larger leaf area. Conversely, shortage of water causes formation of small closely packed cells with high density which explains the high LDMC and LMA at the seasonally dry NWG (Poorter et al., 2009). In addition, the increased toughness improves the survivability of leaves in the harsh environment. LCC was higher at the wet Southern Western Ghats in agreement with Gotsch et al.(2010).

The result that seasonality was more important in defining trait values than that total amount of rainfall is interesting and a novel finding of this study. The fact that in the seasonally dry NWG, leaves formed during monsoon in the presence of abundant water, display traits adaptive to low water condition, suggests that the variation is not just an effect of plasticity. Speculating on the same, individuals in NWG must have adapted to the seasonality of the local climate and are genetically different from the ones in SWG.

Plasticity in traits due to light: The difference between sun and shade leaves normalized by species specific average is referred to as plasticity due to light. As expected, species differed in their plasticity due to light. The traits too differed in their

plasticity to light with Leaf area being the most plastic and LCC being the least plastic trait. However, the range of plasticity across all the species in this study is much lesser compared to the other studies. For example, comparing values from multiple studies (Gratani et al., 2006; Niinemets et al., 2004; Sack et al., 2006) for effect of light on LMA, plasticity varied from 8-58% in other studies to just 8-27% in this study. LDMC, which is known to be one of less plastic traits (Albert et al., 2011), was much lower than LMA at just 2-11% difference between sun and shade leaves. Plasticity in LNC ranged from -10 to 15% and that of LCC was only -6 to 5% (11% difference). Leaf area was the most plastic and had the largest range of variation from -60% to 10%.

As expected, sun leaves had higher LMA and LDMC for sun leaves than the shade leaves. This is due to increased leaf thickness to accommodate a higher number of photosynthetic machinery. In addition, it provides advantage in high light condition by preventing over heating of the leaf. Leaf area was higher for shade leaves indicating the adaptation to improve light interception. Both LCC and LNC lacked consistent directionality in their response to light.

Intraspecific variation in traits was much lesser than the interspecific variation. For example, among the species studied, LMA varied up to five times interspecifically but only up to twice intra-specifically. Contrary to the expectation of a positive relationship, plasticity due to light and intraspecific variation across sites did not have a significant relation. This suggests that across site variation might be due to genetic differences between individuals of the same species.

Variation in plasticity across sites: Species differed in their plasticity for all traits except LMA and LDMC. Most of the variation in leaf nitrogen content is explained by interspecific differences except for a very small contribution from the interactive effect of site and species. Even though LA varies across sites there is considerable variation across species. For LMA, LDMC and LCC, the variation in plasticity was primarily due to differences in site. However, only for LDMC, species responses differed across sites, which contributed to a reasonable amount of total variation. It was interesting to see that plasticity in LMA was halved at the wet SWG. This suggests a major role of water in the way light affects the trait. Leaf carbon content was more varied in the dry NSTR

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Intraspecific variation in trait relationships: LMA and LNC have a strong negative correlation (r^2~0.5) similar to the Leaf Economic Spectrum (Wright et al., 2004) and this trend was consistent for all environmental conditions in this study. The gain in nitrogen and hence photosynthesis for a given increase in LMA did not change across sites or light conditions. But, LNC at a both dry and seasonally dry sites and sun leaves were higher for a given LMA. The higher LNC is suggested as a mechanism to improve water use efficiency at dry sites and maximize photosynthesis at high light condition. The shift in the trait relationship is generally attributed to change in plant community composition (Wright et al., 2001). But in this study, where same species were studied across the three sites, this shift suggests that contribution from intraspecific variation cannot be ignored. As a speculation, the lack of change in slope could mean that there is strong underlying constraint which prevents certain combinations of traits.

The ongoing work includes processing the samples for Leaf Phosphorous Content and also obtain data for stomatal size and density. For each of the traits, the variance will be partitioned across all the hierarchical levels- between leaves, within individual between sun and shade leaves, between individuals at a within a site and between individuals across sites. This will help recognize the levels contributing to most of the variation and help in parametrizing global models. Additionally, it will find use in improving sampling methods.

This field study was planned to understand the effect of climate and light on leaf functional traits. Though site were chosen to have differences in water availability, there are other covariates to site which could effectively have an impact on the traits. Examples of covariates would be differences in cloud cover, cumulative light received annually, differences in herbivory, etc. Future work should focus more on controlled laboratory experiment which can tease apart the covariates and also answers question on mechanistic underpinnings of intraspecific variation. This study would greatly benefit from a thorough community level study for the same sites, which would look at both inter and intraspecific variation and compare them.

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Appendix1

Rainfall ranges studied. The ones in green box are from the tropics.
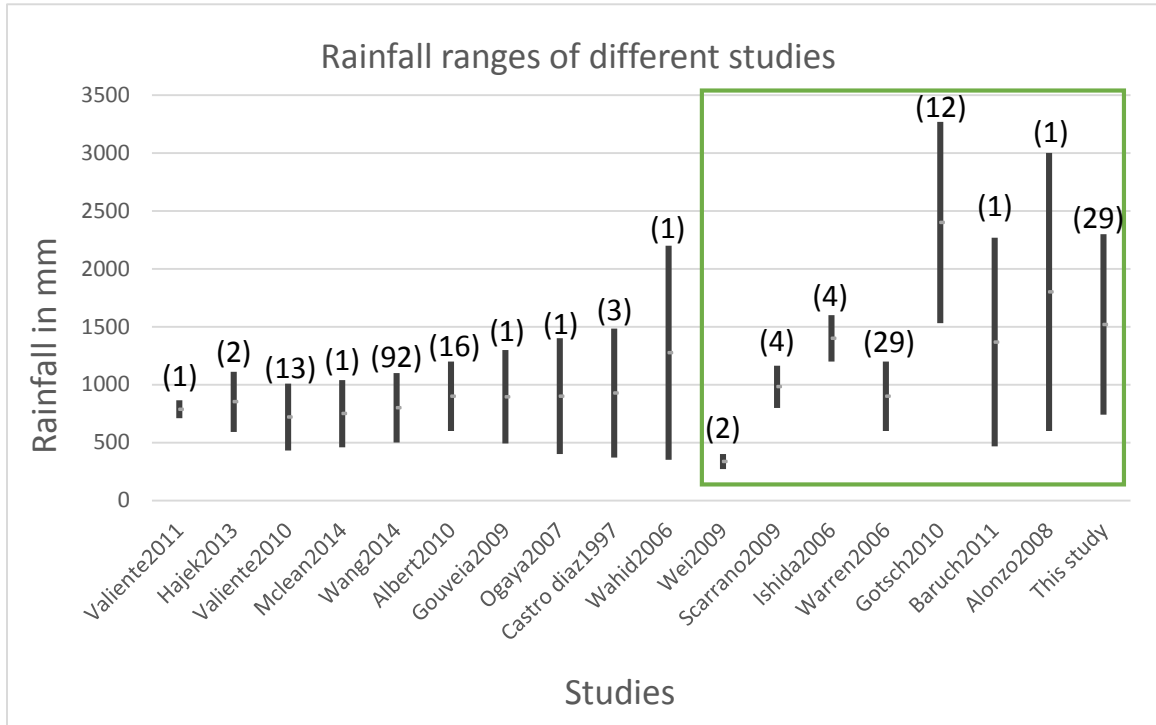
Appendix 2

List of 29 species collected from the three different sites. Absence of a species in a region is represented by '-----'.

	Species	Sp. Code	NSTR	NWG	SWG
1	*Albizzia lebeck*	Aleb	Collected	Collected	-----------------------
2	*Alstonia scholaris*	Ash	-----------------------	Collected	Collected
3	*Atlantia racemosa*	AR	-----------------------	Collected	Collected
4	*Bombax ceiba*	Cba	Collected	-----------------------	Collected
5	*Bridelia retusa*	BR	Collected	Collected	Collected
6	*Carallia brachiata*	CB	-----------------------	Collected	Collected
7	*Careya arborea*	Carb	Collected	Collected	Collected
8	*Cassia fistula*	Cfist	Collected	Collected	Collected
9	*Cassine glauca*	CasG	Collected	Collected	-----------------------
10	*Catenugarum spinosa*	CS	Collected	Collected	Collected
11	*Diospyros montana*	DM	Collected	Collected	-----------------------
12	*Flacourtia indica*	FI	Collected	Collected	-----------------------
13	*Grewia tiliafolia*	GrT	Collected	Collected	Collected
14	*Lagestromia parviflora*	Lpar	Collected	Collected	-----------------------
15	*Leea indica*	Leea	-----------------------	Collected	Collected
16	*Macaranga peltata*	Mpel	-----------------------	Collected	Collected
17	*Mallotus philippensis*	MalP	Collected	Collected	Collected
18	*Mangifera indica*	Mango	Collected	Collected	Collected
19	*Memecylon umbellatum*	Mu	Collected	Collected	Collected
20	*Mitragyna parvifolia*	Mpar	Collected	-----------------------	Collected
21	*Morinda pubescens*	MoP	Collected	Collected	-----------------------
22	*Olea dioica*	Ol	-----------------------	Collected	Collected
23	*Schleichera oleosa*	SchO	Collected	-----------------------	Collected
24	*Sterculia guttata*	Steg	-----------------------	Collected	Collected
25	*Syzygium cumini*	SC	Collected	Collected	Collected
26	*Tectona grandis*	TecG	Collected	Collected	Collected
27	*Terminalia bellerica*	TerB	Collected	Collected	Collected
28	*Terminalia chebula*	TerC	Collected	Collected	-----------------------
29	*Terminalia elliptica*	TerE	Collected	Collected	Collected

Appendix 3

ANOVA tables for effect of species and site on trait values.

a) Leaf mass per unit area (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	28	14.20	0.51	22.81	<0.0001
Site	2	7.72	3.86	173.62	<0.0001
Species X Site	39	3.55	0.09	4.10	<0.0001
Residuals	276	6.14	0.02		

b) Leaf dry matter content (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	28	6.09	0.22	32.27	<0.0001
Site	2	0.56	0.28	41.50	<0.0001
Species X Site	39	1.23	0.03	4.69	<0.0001
Residuals	276	1.86	0.01		

c) Leaf area (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	28	350.06	12.50	156.57	<0.0001
Site	2	9.81	4.91	61.46	<0.0001
Species X Site	39	15.55	0.40	4.99	<0.0001
Residuals	276	22.04	0.08		

d) Leaf Carbon content (data was square transformed)

	df	SS	MS	F value	Pr(>F)
Species	28	7336767	262027.40	15.47	<0.0001
Site	2	1648264	824132.20	48.65	<0.0001
Species X Site	39	3735025	95769.87	5.65	<0.0001
Residuals	276	4675746	16941.11		

e) Leaf Nitrogen content (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	28	25.27	0.9	65.61	<0.0001
Site	2	0.11	0.06	4.08	0.0179
Species X Site	39	2.64	0.07	4.92	<0.0001
Residuals	276	3.8	0.01		

Appendix 4

ANOVA tables for effect of species and site on trait values for a balanced data set for the five traits with 12 species in each site

a) Leaf mass per unit area (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	11	10.268	0.934	37.730	<0.0001
Site	2	4.540	2.270	91.750	<0.0001
Species X Site	22	2.275	0.103	4.180	<0.0001
Residuals	140	3.463	0.025		

b) Leaf dry matter content (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	11	3.839	0.349	45.679	<0.0001
Site	2	0.151	0.076	9.880	<0.0001
Species X Site	22	0.770	0.035	4.582	<0.0001
Residuals	140	1.070	0.008		

c) Leaf area (data was log transformed)

	Df	SS	MS	F value	Pr(>F)
Species	11	180.130	16.375	200.381	<0.0001
Site	2	1.040	0.520	6.358	0.0022
Species X Site	22	8.140	0.370	4.529	<0.0001
Residuals	140	11.440	0.082		

d) Leaf Carbon content (data was square transformed)

	df	SS	MS	F value	Pr(>F)
Species	11	4539960	412724	27.666	<0.0001
Site	2	424895	212447	14.241	<0.0001
Species X Site	22	2548195	115827	7.764	<0.0001
Residuals	140	2088563	14918		

e) Leaf Nitrogen content (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	11	15.961	1.451	86.692	<0.0001
Site	2	0.054	0.027	1.618	0.2020
Species X Site	22	1.392	0.063	3.781	<0.0001
Residuals	140	2.343	0.017		

Appendix 5

ANOVA results for comparison between the sites with different rainfall but seasonal NSTR – NWG for the five traits.

a) Leaf mass per unit area (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	17	11.36	0.67	40.43	<0.0001
Site	1	0.02	0.02	1.12	0.2926
Species X Site	17	0.66	0.04	2.37	0.0031
Residuals	144	2.38	0.02		

b) Leaf dry matter content (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	17	3.41	0.2	31.09	<0.0001
Site	1	0.01	0.01	1.91	0.1694
Species X Site	17	0.46	0.03	4.17	<0.0001
Residuals	144	0.93	0.01		

c) Leaf area (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	17	136.71	8.04	93.85	<0.0001
Site	1	<0.00	<0.00	<0.00	0.9638
Species X Site	17	3.68	0.22	2.53	0.0015
Residuals	144	12.34	0.09		

d) Leaf Carbon content (data was square transformed)

	df	SS	MS	F value	Pr(>F)
Species	17	4382138	257772.9	12.16	<0.0001
Site	1	87860.33	87860.33	4.14	0.0436
Species X Site	17	2357906	138700.3	6.54	<0.0001
Residuals	144	3053735	21206.5		

e) Leaf Nitrogen content (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	17	19.45	1.14	77.3	<0.0001
Site	1	0.19	0.19	12.5	0.0005
Species X Site	17	0.91	0.05	3.6	<0.0001
Residuals	144	2.13	0.01		

Appendix 6

ANOVA results for comparison between sites with similar mean rainfall and differing seasonality, NWG-SWG for five traits.

a) Leaf mass per unit area (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	18	7.07	0.39	14.15	<0.0001
Site	1	6.25	6.25	225.11	<0.0001
Species X Site	18	1.70	0.09	3.40	<0.0001
Residuals	151	4.19	0.03		

b) Leaf dry matter content (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	18	3.67	0.20	28.47	<0.0001
Site	1	0.42	0.42	59.11	<0.0001
Species X Site	18	0.59	0.03	4.57	<0.0001
Residuals	151	1.08	0.01		

c) Leaf area (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	18	252.61	14.03	179.86	<0.0001
Site	1	1.67	1.67	21.39	<0.0001
Species X Site	18	8.86	0.49	6.31	<0.0001
Residuals	151	11.78	0.08		

d) Leaf Carbon content

	df	SS	MS	F value	Pr(>F)
Species	18	0.152	0.0084	19.31	<0.0001
Site	1	0.020	0.0204	47.88	<0.0001
Species X Site	18	0.075	0.0042	9.63	<0.0001
Residuals	151	0.066	0.0004		

e) Leaf Nitrogen content (data was log transformed)

	df	SS	MS	F value	Pr(>F)
Species	18	12.45	0.69	53.20	<0.0001
Site	1	0.03	0.03	2.06	0.1534
Species X Site	18	1.44	0.08	6.15	<0.0001
Residuals	151	1.96	0.01		

Appendix 7

ANOVA table for effect of species and site on plasticity due to light for the five traits.

a) Leaf mass per unit area

	df	SS	MS	F value	Pr(>F)
Species	28	0.68	0.02	1.35	0.1185
Site	2	1.54	0.77	43.18	<0.0001
Species X Site	39	1.16	0.03	1.66	0.0114
Residuals	276	4.94	0.02		

b) Leaf dry matter content

	df	SS	MS	F value	Pr(>F)
Species	28	0.18	0.01	1.01	0.4516
Site	2	0.04	0.02	3.38	0.0355
Species X Site	39	0.52	0.01	2.10	<0.0003
Residuals	276	1.76	0.01		

c) Leaf area

	df	SS	MS	F value	Pr(>F)
Species	28	7.58	0.27	3.22	<0.0001
Site	2	0.52	0.26	3.08	0.0475
Species X Site	39	5.68	0.14	1.73	0.0064
Residuals	276	23.17	0.08		

d) Leaf Carbon content

	df	SS	MS	F value	Pr(>F)
Species	28	0.05	0.0017	3.32	<0.0001
Site	2	0.04	0.0219	42.91	<0.0001
Species X Site	39	0.06	0.0015	2.96	<0.0001
Residuals	276	0.14	0.0005		

e) Leaf Nitrogen content

	df	SS	MS	F value	Pr(>F)
Species	28	0.70	0.02	2.30	0.0003
Site	2	0.10	0.05	4.67	0.0101
Species X Site	39	0.75	0.02	1.76	0.0050
Residuals	276	3.02	0.01		

Appendix 8

ANOVA tables for effect of species and site on plasticity due to light for the five traits for a balanced data set traits with 12 species in each site

a) Leaf mass per unit area

	df	SS	MS	F value	Pr(>F)
Species	11	0.284	0.0258	1.457	0.1546
Site	2	0.999	0.4999	28.191	<0.0001
Species X Site	22	0.626	0.0285	1.605	0.0532
Residuals	140	2.482	0.0177		

b) Leaf dry matter content

	df	SS	MS	F value	Pr(>F)
Species	11	0.0618	0.0056	0.968	0.4783
Site	2	0.0475	0.0237	4.087	0.0188
Species X Site	22	0.3084	0.0140	2.414	0.0010
Residuals	140	0.8130	0.0058		

c) Leaf area

	df	SS	MS	F value	Pr(>F)
Species	11	3.662	0.3329	3.982	<0.0001
Site	2	0.882	0.4411	5.276	0.0061
Species X Site	22	2.891	0.1314	1.572	0.0613
Residuals	140	11.705	0.0836		

d) Leaf Carbon content

	df	SS	MS	F value	Pr(>F)
Species	11	0.01709	0.0015	2.560	0.0055
Site	2	0.0171	0.0157	25.849	<0.0001
Species X Site	22	0.0361	0.0016	2.705	0.0002
Residuals	140	0.0849	0.0006		

e) Leaf Nitrogen content

	df	SS	MS	F value	Pr(>F)
Species	11	0.3612	0.0328	3.112	0.0008
Site	2	0.0387	0.0193	1.833	0.1637
Species X Site	22	0.4103	0.0186	1.767	0.0256
Residuals	140	1.4773	0.0105		

Appendix 9

Ranges of interspecific variation in traits and comparison with global data sets.

Trait	Range in this study	Global range	Reference
LMA (g/m^2)	55-250	25-370 (2474 species)	GLOPNET; Tropical trees
LNC (%)	0.91-3.73	0.48-4.28 (2413 species)	GLOPNET; Tropical trees
LCC (%)	44.7-53.7	40-54 (7856 species)	TRY database; all data
LDMC (mg/g)	256-586	NA	NA
LA (cm^2)	9-1287	NA	NA