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Supplementary material

SUPPLEMENTARY MATERIAL - APPENDIX 1. MATERIALS AND METHODS

Leaf traits

For each leaf sample, we measured five leaf functional traits reflecting different aspects of plant strategies and leaf function. LMA, the foliar dry mass per unit fresh area (g/m^2), reflects the amount of biomass investment per unit of light capture area. LDMC, the ratio of a leaf's dry mass to its water-saturated mass (g/g), reflects the tradeoff in investing resources in structural tissues versus liquid-phase processes. LDMC has been argued to be the central variable underpinning correlations among the traits in the leaf economic spectrum (Shipley et al. 2006) and has been shown to be a good proxy for leaf tissue density (Vile et al. 2005). Leaf nitrogen content (LNC), the fraction of a leaf's total dry weight accounted by nitrogen (g/g), reflects photosynthetic capacity because foliar nitrogen is mostly present in RUBISCO and chlorophyll (Evans 1989). In leaves, nitrogen is also found in inducible anti-herbivory compounds such as alkaloids but they usually constitute a small amount of total nitrogen in healthy leaves. LMA, LDMC and LNC are correlated because they are part of the same ecological strategy dimension, known as the leaf economic spectrum. On one end of the spectrum, leaves have a high photosynthetic rate (high LNC), which usually entails thinner and/or less dense leaves (low LMA, low LDMC), as well as more vulnerable and shorter lived leaves. On the other end of the spectrum, leaves have a low photosynthetic rate (low LNC), which involves thicker (high LMA, high LDMC), more durable and longer lived leaves (Reich et al. 1999, Wright et al. 2004).

Leaf carbon content (LCC), the fraction of a leaf's total dry weight accounted by carbon (g/g), mostly reflects the leaves' investment in structural support (Niinemets et al. 2007) and mechanical defense from herbivory (Coley and Barone 1996, Lucas et al. 2000). In the leaf, carbon is primarily found in the cell wall in the forms of lignin, cellulose, hemicellulose, and in carbohydrate compounds such as sugars and starch. Lignin, cellulose and hemicellulose are cell wall components that provide structural support and mechanical protection to the leaf. Part of the leaf carbon content is also found in proteins, where it constitutes ca. 53% of protein's weight (Vertregt and Devries 1987).

Leaf Area, the projected area of one side of the leaf blade (cm^2), is an architectural trait that is part of a strategy dimension known as Corner's Rules (White 1983, Brouat et al. 1998, Olson et al. 2009). Corner (Corner 1949) described two architectural rules: (1) the larger the plant appendage (fruit, flower, leaf), the larger the twig or branch to which it is attached and (2) the more highly branched the twigs, the smaller their sizes. These allometric relationships have been suggested by Olson *et al*. (2009)

to result from the combined effects of metabolic scaling (West et al. 1999) and constant carbon assimilation rate per unit crown area (Enquist et al. 1999), together leading to a trade-off between the mechanical support and transport functions of the stems and leaves. Plant architecture is ecologically important because it affects three fundamental plant functions: mechanical support, light capture through leaf spatial arrangement, and reproduction through flower display and seed dispersal (Niklas 1994). Finally, while Leaf Area is a component of LMA, it is well established as an independent axis of variation among species (e.g. Westoby et al. 2002, Poorter and Rozendaal 2008).

Sampling Design

The sampling design spanned six important ecological scales: (1) among leaves within a stratum; (2) between strata within a tree; (3) among trees within a species; (4) among species; (5) among plots within a site and (6) among sites within a biome (Figure A1). Three sites were sampled: Parque Natural Metropolitano (PNM) located close to the Pacific coast, Barro Colorado Island (BCI) located on the Panama Canal and Parque Natural San Lorenzo located on the Atlantic coast (PNSL). These sites are located in lowland tropical rainforests along the Panama Canal and follow a strong precipitation and seasonality gradient. Further details on the study sites are provided in Table A1. Four (in PNM) or eight (in BCI and PNSL) 400 m^2 plots, systematically located 60-80 m apart center to center, were sampled at each of the three sites. Each site covers ca. 1 (PNM) to ca. 3 ha in area (BCI and PNSL). Within each plot, all trees with a diameter at breast height (dbh) greater or equal to 10 cm were sampled. For each tree, three healthy and fully mature leaves were randomly sampled from a branch collected from each of the sun and shade stratum, yielding a total of 1910 leaf samples across 124 species (See Table A3 for species list). For elemental analysis, petioles were removed and each leaf was homogenized and ground to a fine powder using a Thomas Wiley Mini-Mill. The carbon and nitrogen content of the leaf blades were determined on 1.0-2.0 mg of ground leaf material using a Fison EA 1108 CHNS-O Elemental Analyzer. Elemental analyses were standardized using acetanilide, atropine and BBOT. All trait values were transformed using the natural logarithm to improve normality.

The following describes which drivers of phenotypic variation are associated with each of the scales in this study. This results from the specifics of the sampling design.

Differences among *leaves* within a stratum reflect developmental instability, the ontogenetic effects of metamer and module position along the plant and potentially the plastic response to micro-environmental gradients. To minimize trait variation due to leaf age we sampled leaves that are all fully mature but not senescing.

Differences among *strata* within a tree mostly reflect the plastic response to light because strata were defined based on their exposure to sunlight. Developmental instability and other micro-environmental gradients can also cause variation among strata.

Differences among *trees* within a species reflect developmental instability, tree age, sexual genetic mixing, and plastic and filtering responses to micro-environmental gradients. To minimize the effect of tree age on trait measurements, we only sampled mature trees (dbh >10cm). Unfortunately, the high alpha and beta diversities of the study system do not allow us to measure variation among populations of a species.

Differences among *species* mainly reflect genetic differences resulting from adaptive evolution and drift. In this study, due to the high species richness and turnover, the species scale is mostly nested within sites and partly nested within plots. This means that variance at the species scale also somewhat reflects the environmental effects due to differences among sites. However, Table A8 shows that the environment effect on species level variance is minor: When removing the species level from the analyses, the species level variance gets reassigned to the tree, plot and species levels and the majority of the variance originally attributed to the species level gets re-attributed to the tree level (66% for LDMC to 89% for Leaf Area) and the site and plot level variances increase but modestly.

Among plots within a site, the average Sorensen' similarity indices (Chao et al. 2005) are 0.33 for PNM, 0.13 for BCI and 0.23 for PNSL. Among sites, the indices are 0.02 between PNM and BCI, 0.26 between BCI and PNSL and 0.00 between PNM and PNSL. Despite this high species turnover, the species level does not fall entirely within the ecological hierarchy presented in Figure A1. Thus, in the analyses the species scale was crossed with the site scale (see the statistical analyses section for full details on the structure of the statistical model).

In this study, the *plots* were located within a habitat with no noticeable environmental gradients among them. Differences due to species composition are accounted for at the species level and the effects of differences in species composition is removed from the plot-level variance. In the study, plot variance therefore reflects undetectable differences in the environment and the natural variability in trait composition that occurs within habitats.

Since three study *sites* are located along a strong precipitation gradient, differences among them reflect community-level ecological filtering along a climatic gradient. Although there is a strong species turnover among sites, differences among species is accounted for at the species level and the effects of species composition is removed from the site-level variance.

Statistical analyses

Note that we do not observe meaningful qualitative changes between this model crossing the species and site hierarchies and the model used in Messier *et al*. (2010), which nests the species level within plots. Further, Table A8 shows that removing the species level from the analysis mainly leads to an increase in the variance at the tree level, with little change in the variance at the site level. This indicates that despite the species turnover across sites, the model is largely parses out species level variance from site-level variance.

To calculate confidence intervals, tor each trait we created 500 simulated datasets by resampling the data with replacement. We then calculated the variance components on each simulated dataset. For each scale, the 95% confidence intervals were calculated from the results of the 500 variance components analyses. Palm fronds were too large to collect intact, and hence were excluded from analyses for Leaf Area. Some leaves were too small to provide sufficient material for the stoichiometry analyses and were also excluded from the statistical analyses. Sample size was thus n=1890 for LMA, n=1896 for LDMC, n=1860 for LNC, LCC and 1784 for Leaf Area.

We used the *rda()* function of the *vegan* package in R (R Development Core Team 2011). We used the correlation matrix of the data in order to give each ecological scale an equivalent weight. Last, we calculated a traits dissimilarity index for their variance structure across scales by measuring the Euclidian distances between trait pairs along principal components 1 and 2. We weighted the distance between trait pairs along each principal component by the principal component's eigenvalue.

Appendix 1 References

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SUPPLEMENTARY MATERIAL - APPENDIX 2. SUPPLEMENTARY TABLES AND FIGURES

Table A1. Description of the three study sites located along the Panama Canal. PNM: Parque Natural San Lorenzo, BCI: Barro Colorado Island, PNSL: Parque Natural San Lorenzo, MAP: Mean Annual Precipitation, MDSL: Mean Dry Season Length. Information from: 1 – (Smithsonian Tropical Research Institute 2007), 2- (Santiago and Mulkey 2005), 3- (Santiago et al. 2004), 4- (Condit et al. 2004), 5- (Leigh et al. 2004). * : calculated as the mean interval during which potential evapotranspiration (PET) exceeds rainfall. ** : species with individuals with stems ≥10 cm in diameter.

Site	**Location**	**MAP** (mm)	**MDSL** * (days)	**Parent material**	**Elevation** (m)	**Richness** (sp/ha)	**Tree density**** (#/ha)
PNM	[1]8°59'N 79°33'W	[1]1850	[2]129	[2]Volcanic	[3]60	[3]36	[3]318
BCI	[1]9°10'N 79°51'W	[1]2620	[4]118	[5]Volcanic	[5]40	[5]91	[5]429
PNSL	[1]9°17'N 79°58'W	[1]3020	[2]102	[2]Sedimentary	[3]140	[3]87	[3]659

Table A2. Comparison of the Minimum, Maximum, Mean, Max/Min and Coefficient of Variation of the five leaf functional traits studied with the GLOPNET dataset. The statistics presented are for not-transformed data. Of the five study traits, only LMA and LNC values are available in the GLOPNET dataset. Leaf Mass per Area, LMA ($g \cdot m^{-2}$); Leaf Dry Matter Content, LDMC ($g \cdot g^{-1}$); Leaf Nitrogen Content, LNC ($g \cdot g^{-1} \cdot 100^{-1}$); Leaf Carbon Content, LCC ($g \cdot g^{-1} \cdot 100^{-1}$), Leaf Area, Area ($m^2$).

	PANAMA					**GLOPNET**	
	LMA	**LDMC**	**LNC**	**LCC**	**Leaf Area**	**LMA**	**LNC**
Min	22.29	0.08	0.68	33.16	0.0002	14.45	0.25
Max	235.6	0.72	5.96	58.03	0.39	1514	6.35
Variance	970	0.005	0.49	13.97	0.0008	14028	0.96
Max/Min	10.5	8.53	9.61	1.75	1938	104	25.40
CV (sd/mean)	0.36	0.20	0.33	0.08	2.19	0.93	1.05

Table A3. Species List

Alibertia edulis	*Guarea guidonia*	*Picramnia latifolia*
Alseis blackiana	*Guatteria dumetorum*	*Piper reticulatum*
Anacardium excelsum	*Guazuma ulmifolia*	*Pittoniotis trichantha*
Andira inermis	*Guettarda foliacea*	*Platypodium elegans*
Apeiba membranacea	*Hamelia axillaris*	*Poulsenia armata*
Aspidosperma cruentum	*Hasseltia floribunda*	*Pourouma bicolor*
Aspidosperma spruceanum	*Heisteria acuminata*	*Pouteria reticulata*
Astrocaryum standleyanum	*Heisteria concinna*	*Protium costaricense*
Astronium graveolens	*Herrania purpurea*	*Protium panamense*
Beilschmiedia pendula	*Hieronyma alchorneoides*	*Protium tenuifolium*
Brosimum alicastrum	*Hirtella triandra*	*Psychotria horizontalis*
Brosimum guianensis	*Humiriastrum diguense*	*Pterocarpus rohrii*
Brosimum utile	*Hybanthus prunifolius*	*Quararibea asterolepis*
Carapa guianensis	*Inga nobilis*	*Randia armata*
Cassipourea elliptica	*Inga pezizifera*	*Simarouba amara*
Castilla elastica	*Inga sapindoides*	*Sloanea meianthera*
Cecropia insignis	*Jacaranda copaia*	*Sloanea terniflora*
Cecropia obtusifolia	*Lacistema aggregatum*	*Socratea exorrhiza*
Cespedesia spathulata	*Lacmellea panamensis*	*Spondias mombin*
Chamguava schippii	*Lindackeria laurina*	*Symphonia globulifera*
Chimarrhis parviflora	*Luehea seemannii*	*Tabebuia guayacan*
Chrysophyllum argenteum	*Manilkara bidentata*	*Tabernaemontana arborea*
Cinnamomum triplinerve	*Maquira guianensis*	*Tachigali versicolor*
Cordia alliodora	*Maranthes panamensis*	*Tapirira guianensis*
Cordia bicolor	*Marila laxiflora*	*Terminalia oblonga*
Croton billbergianus	*Matayba apetala*	*Theobroma bernoullii*
Cupania scrobiculata	*Miconia elata*	*Tovomita longifolia*
Dendropanax arboreus	*Miconia ligulata*	*Trattinnickia aspera*
Desmopsis panamensis	*Miconia minutiflora*	*Trichilia poeppigii*
Diospyros artanthifolia	*Miconia sp*	*Trichilia tuberculata*
Dussia sp	*Mortoniodendron anisophyllum*	*Turpinia occidentalis*
Eugenia coloradoensis	*Nectandra purpurea*	*Unonopsis panamensis*
Eugenia nesiotica	*Ochroma pyramidale*	*Unonopsis pittieri*
Eugenia oerstediana	*Ocotea cernua*	*Virola elongata*
Faramea occidentalis	*Ocotea dendrodaphne*	*Virola multiflora*
Ficus insipida	*Ocotea ira*	*Virola sebifera*
Ficus maxima	*Oenocarpus mapora*	*Virola surinamensis*
Garcinia intermedia	*Ormosia coccinea*	*Vochysia ferruginea*
Garcinia madruno	*Palicourea guianensisv*	*Xylopia macrantha*
Guapira standleyana	*Perebea xanthochyma*	

Table A4. Correlations and variances among the five study traits. Trait variances are in bold along the diagonal, the Pearson correlation coefficients (r), are located below the diagonal and statistical significance of the correlations (p-values) are above the diagonal. Significant correlations are also marked with a star. Each data point is a leaf-level measurement. Data were natural log transformed.

	LMA	**LDMC**	**LNC**	**LCC**	**Leaf Area**
LMA	**0.131**	< 2.2e-16	< 2.2e-16	< 2.2e-16	0.227
LDMC	0.585*	**0.046**	< 2.2e-16	< 2.2e-16	5.06e-15
LNC	-0.526*	-0.411*	**0.092**	0.017	0.829
LCC	0.292*	0.262*	-0.055*	**0.006**	< 2.2e-16
Leaf Area	0.029	-0.185*	0.005	-0.252*	**1.062**

Table A5. Variance partitioning analyses of the five leaf traits giving the percentage of total variance explained by each scale and their confidence intervals (0.025 and 0.975 quantiles) calculated by bootstrapping. Data was normalized using natural log transformation.

Scale	LMA	LDMC	LNC	LCC	Leaf Area
Leaf & Error	11 (7-10)	17 (10-17)	11 (7-9)	10 (6-10)	7 (5-6)
Strata	17(15-21)	11 (10-17)	6 (6-11)	8(8-14)	6 (6-9)
Tree	21 (18-25)	13 (10-20)	16 (13-19)	12 (5-14)	7 (5-9)
Species	30 (26-34)	44 (39-49)	44 (40-48)	45 (42-49)	79 (77-80)
Plot	0 (0-1)	0 (0-1)	3 (2-5)	3 (2-4)	0 (0-1)
Site	21 (19-25)	15 (11-19)	20 (18-24)	22 (20-24)	1 (1-2)

Table A6. Loadings of Principal Components Analysis on variance component table

		PC1	PC2
Proportion of total variance		0.58	0.27
Loadings	Leaf &		
Error		0.29	0.25
	Strata	042	0.42
	Tree	0.49	-0.02
	Species	- 0.53	0.10
	Plot	0.01	- 0.78
	Site	0.46	- 0.37

Table A7. Trait standardized major axis regressions (*sma* function, *smatr* package, R software) for three leaf economic spectrum traits among species in three communities spanning a precipitation and seasonality gradient along the panama canal (Table A1). PNM n=10, BCI n=58, SANLO n=35. r-values, p-values and slopes are given only for the significant correlations.

Traits	**Community**		
	PNM	**BCI**	**PNSL**
LMA- LNC	n.s.	r= 0.49 slope= -1.06	r= 0.72 slope = -1.04
LMA- LDMC	n.s.	r= 0.55 slope = 1.58	n.s.
LNC-LDMC	n.s.	r= 0.53 slope = - 1.49	n.s.

Table A8. Variance partitioning analyses of the five leaf traits without the species scale.

Scale	LMA	LDMC	LNC	LCC	Leaf Area
Leaf & Error	11	16	11	9	9
Strata	17	11	6	9	6
Tree	43	52	50	46	77
Plot	0	3	6	7	6
Site	29	19	26	31	1

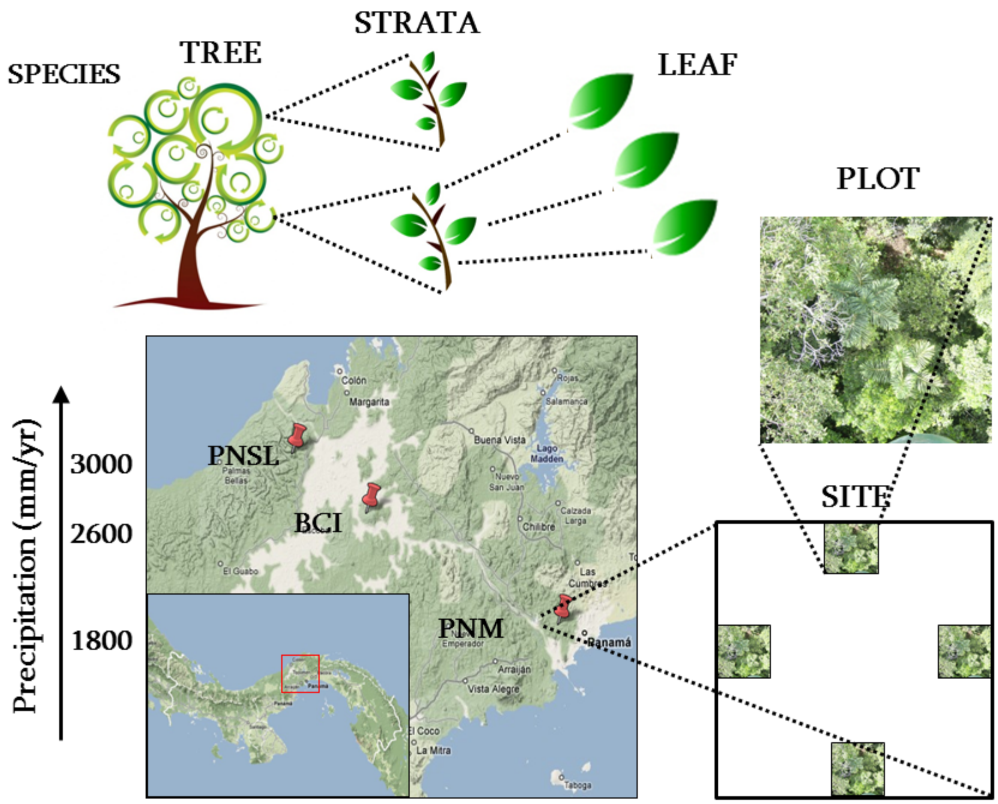

Figure A1. Sampling design illustrating the six ecological scales and their nested structure.

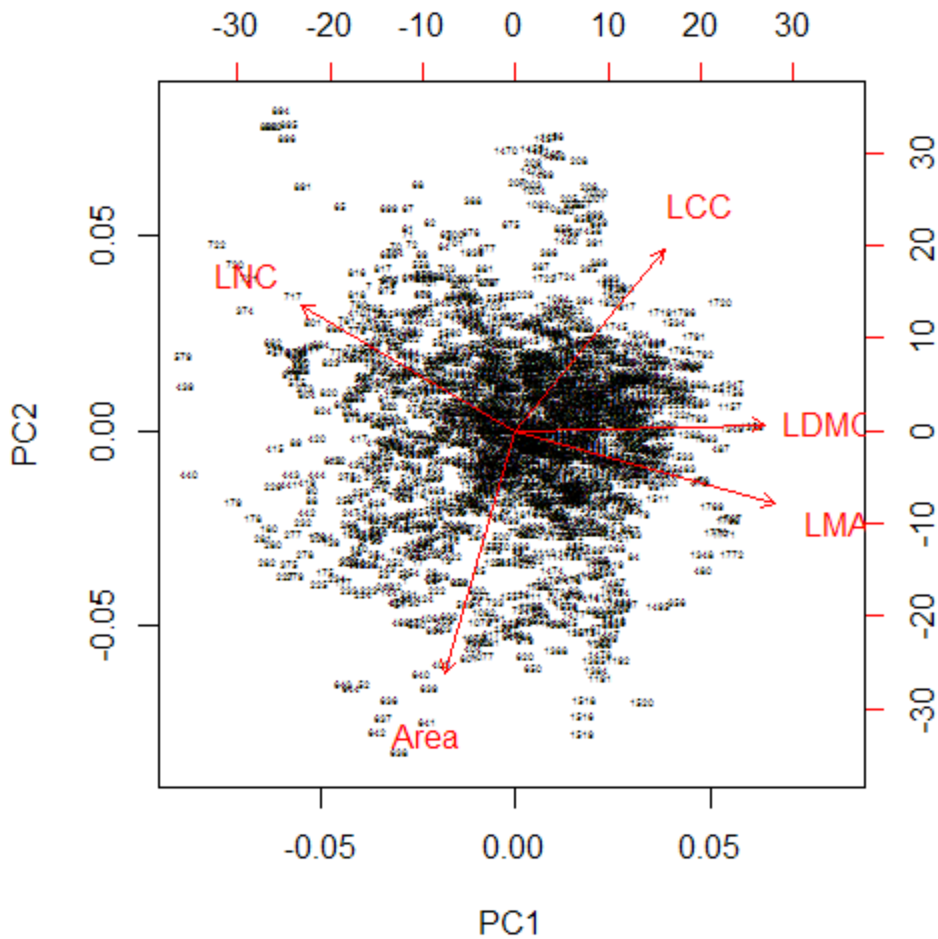

Figure A2 – Principal component analysis of the five leaf traits at the leaf level. Data natural log transformed and standardized