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Using functional traits and phylogenetic trees to examine the assembly of tropical tree communities

Christopher Baraloto[1,2], Olivier J. Hardy[3], C. E. Timothy Paine[4,5], Kyle G. Dexter[6], Corinne Cruaud[7], Luke T. Dunning[8,9], Mailyn-Adriana Gonzalez[6,10], Jean-François Molino[11], Daniel Sabatier[11], Vincent Savolainen[8,12] and Jerome Chave[6]*

[1]*INRA, UMR "Ecologie des Forêts de Guyane", 97387 Kourou Cedex, French Guiana;* [2]*Department of Biology, University of Florida, Gainesville, FL 32611, USA;* [3]*Evolutionary Biology and Ecology, CP160/12, Faculté des Sciences, Université Libre de Bruxelles, av. F.D. Roosevelt 50, 1050 Brussels, Belgium;* [4]*AgroParisTech, UMR "Ecologie des Forêts de Guyane", 97387 Kourou Cedex, French Guiana;* [5]*Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland;* [6]*Université Paul Sabatier, CNRS, UMR 5174 Laboratoire Evolution et Diversité Biologique, 31062 Toulouse, France;* [7]*Génoscope, Commissariat à L'Energie Atomique–Institut de Génomique, 91057 Evry Cedex, France;* [8]*Imperial College London, Silwood Park Campus, Ascot SL5 7PY, UK;* [9]*Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand;* [10]*Departamento de Ciencias Biológicas, Universidad de los Andes, Carrera 1 N 18A-10, Bogotá, Colombia;* [11]*IRD, UMR AMAP, TA-A51/PS2, 34398 Montpellier Cedex 5, France; and* [12]*Royal Botanic Gardens, Kew, Richmond TW9 3DS, UK*

Summary

1. Niche theory proposes that species differences underlie both coexistence within communities and the differentiation in species composition among communities via limiting similarity and environmental filtering. However, it has been difficult to extend niche theory to species-rich communities because of the empirical challenge of quantifying niches for many species. This has motivated the development of functional and phylogeny-based approaches in community ecology, which represent two different means of approximating niche attributes.

2. Here, we assess the utility of plant functional traits and phylogenetic relationships in predicting community assembly processes using the largest trait and phylogenetic data base to date for any set of species-rich communities.

3. We measured 17 functional traits for all 4672 individuals of 668 tree species co-occurring in nine tropical rain forest plots in French Guiana. Trait variation was summarized into two ordination axes that reflect species niche overlap.

4. We also generated a dated molecular phylogenetic tree based on DNA sequencing of two plastid loci (*rbcL* and *matK*) comprising 97% of the individuals and 91% of the species in the plots.

5. We found that, on average, co-occurring species had greater functional and, to a lesser extent, phylogenetic similarity than expected by chance.

6. We also found that functional traits and their ordination loadings showed significant, albeit weak, phylogenetic signal, suggesting that phylogenetic distance provides pertinent information on niche overlap in tropical tree communities.

7. *Synthesis.* We provide the most comprehensive examination to date of the relative importance of environmental filtering and limiting similarity in structuring tropical tree communities. Our results confirm that environmental filtering is the overriding influence on community assembly in these species-rich systems.

Key-words: competition, determinants of plant community diversity and structure, environmental filtering, French Guiana, functional traits, limiting similarity, niche, phylogenetic signal, tropical forests

*Correspondence author. E-mail: chave@cict.fr

Introduction

Understanding the determinants of species coexistence in complex and species-rich ecological communities is a fundamental question in ecology (Chesson 2000; Ricklefs 2004). Competition theory predicts that if two species have identical niches, either one species will exclude the other, causing spatial and/or temporal partitioning (Diamond 1975's checkerboard distribution, Gotelli & McCabe 2002; Chao *et al.* 2005); or selective pressure will eventually result in character displacement (Dayan & Simberloff 2005). However, the physical environment also imposes ecological and evolutionary constraints that create an 'ecological filter' such that species with similar ecological requirements are found in similar environments, a pattern referred to as spatial niche clustering (Grime 1979; Keddy 1992; Myers & Harms 2009). Ultimately, patterns of species coexistence may depend on how both biotic interactions and environmental filtering act over ecological and evolutionary time-scales (Webb *et al.* 2002). Alternatively, species may drift neutrally in abundance, yet co-occur over long periods of time (Hubbell 2001), or biotic interactions and filtering may balance each other to produce seemingly random, or neutral, patterns (Purves & Pacala 2005). Two powerful approaches have been developed recently to test these predictions. Both are based on comparing the similarity of species within and among local communities.

Plant functional traits are often used as proxies to determine whether species have different ecological strategies for reproduction and resource capture (Keddy 1992; McGill *et al.* 2006). If the niches of two species overlap, then it is expected that these species are also similar with respect to a range of physiological, reproductive and defensive functional traits (Díaz, Cabido & Casanoves 1998; Wright *et al.* 2004; Westoby & Wright 2006). Further, it is often assumed that a limited number of functional traits may be sufficient to describe the performance and distribution of species across environmental gradients (Díaz *et al.* 2004; McGill *et al.* 2006; Westoby & Wright 2006; Suding *et al.* 2008). Approaches based on functional traits have been used to demonstrate the importance of environmental filtering in structuring diverse ecological communities, including fish (Bellwood *et al.* 2006; Mouillot, Dumay & Tomasini 2007; Ingram & Shurin 2009; Villéger *et al.* 2010), tropical trees (Kraft, Valencia & Ackerly 2008; Paine *et al.* 2011) and temperate woody plants (Díaz *et al.* 2004; Cornwell, Schwilk & Ackerly 2006; Cornwell & Ackerly 2009). However, the trait-based approach is constrained by the number of pertinent traits that may be measured with accuracy (Poorter *et al.* 2009; Baraloto *et al.* 2010a; Kattge *et al.* 2011; Markesteijn *et al.* 2011), and by the ability to measure intraspecific trait variability (Baraloto *et al.* 2010b; Albert *et al.* 2012). This may explain why functional traits have been found to be relatively poor correlates of demographic parameters (Poorter *et al.* 2008; Kraft *et al.* 2010). Furthermore, the role of functional traits in shaping community assembly processes is difficult to predict from patterns. For instance, research on plant invasions reveals that invasive species seldom differ functionally from their native relatives (e.g. Scharfy *et al.* 2011). This led Mayfield & Levine (2010) to question the assumption that species differences have a simple and predictable imprint on the structure of ecological communities.

Over the past decade, DNA data for phylogeny reconstruction have become relatively easy to obtain (McPeek & Brown 2007), and they have provided useful information on the biogeography and ecology of many groups of organisms. A phylogenetic test of theories of species coexistence has consequently been developed (Grandcolas 1998; Webb 2000). This approach is based on the assumption that phylogenetic proximity among species may be used as a surrogate for niche overlap (Harvey & Pagel 1991; Silvertown, Franco & Harper 1997; Webb 2000; Webb *et al.* 2002; Cavender-Bares *et al.* 2004; Losos 2008). Phylogenetic trees may indeed complement functional trait measures for potentially difficult-to-measure traits, if these traits are phylogenetically correlated (e.g. seed mass, Moles *et al.* 2005). The phylogenetic approach has spawned a novel merging of community ecology and systematics (Webb *et al.* 2002; Cavender-Bares *et al.* 2009; Lavergne *et al.* 2010; Swenson 2011), in addition to statistical methods and software for the integration of phylogenetic trees into ecological analyses (Webb & Donoghue 2005; Hardy & Senterre 2007; Helmus *et al.* 2007; Webb, Ackerly & Kembel 2008; Pausas & Verdú 2010; Eastman, Paine & Hardy 2011). The phylogeny-based approach has confirmed the importance of environmental filtering in community assembly (Pennington, Lavin & Oliveira-Filho 2009; Swenson & Enquist 2009; Kraft & Ackerly 2010; Uriarte *et al.* 2010; Hardy *et al.* 2012), as well as the role of plant–pathogen interactions in promoting species coexistence via negative density dependence (Gilbert & Webb 2007; Bagchi, Press & Scholes 2010; Gonzalez *et al.* 2010). This approach has also been instrumental to readdress classical issues in island biogeography (Emerson & Gillespie 2008; Graham & Fine 2008; Schaefer *et al.* 2011) and at the regional scale in mainland environments (Heard & Cox 2007; Jabot & Chave 2009; Moen, Smith & Wiens 2009; Leibold, Economo & Peres-Neto 2010; Morlon *et al.* 2011).

However, the phylogeny-based approach is predicated on the assumption that phylogenetic distances are indicative of ecological niche differences. This hypothesis has more often been assumed than tested (reviewed in Vamosi *et al.* 2009; but see Cavender-Bares *et al.* 2004; Swenson & Enquist 2009; Kraft & Ackerly 2010). Indeed, phylogenetic distance may be a poor proxy for niche similarity if niches show high rates of evolution or if niches represent convergent evolution. Two recent meta-analyses contest the widespread belief that competition may be most intense among closely related species and over small spatial scales (Vamosi *et al.* 2009's Darwin-Hutchinson zone). Cahill *et al.* (2008) found little evidence that more closely related plant species compete more strongly (see also Mayfield & Levine 2010); and Cadotte, Hamilton & Murray (2009) found no evidence for phylogenetic clustering of invasion success at the landscape scale. The power of the phylogeny-based approach can also be limited by the support for the underlying phylogenetic hypothesis. For example, many recent analyses with plants assume a star-shaped phylogeny (polyto-

my) below the family level (e.g. using the tree from Davies *et al.* 2004), thereby masking all patterns that occur within families (Cavender-Bares *et al.* 2004; Fine *et al.* 2005; Slingsby & Verboom 2006; Kursar *et al.* 2009).

Despite considerable promise, it is thus apparent that both functional and phylogenetic approaches to understanding community assembly have limitations. Therefore, tests of community assembly rules should not only integrate many functional traits measured across multiple individuals per species, but they should also incorporate well-resolved molecular phylogenetic trees developed for the same species present in the communities under study. Some recent publications have begun to address this issue, focusing on the role of species niche differences and competition as community assembly processes (Mayfield & Levine 2010), and developing statistical frameworks to better integrate trait-based and phylogenetic information in tests of community assembly (Kühn, Nobis & Durka 2009; Eastman, Paine & Hardy 2011; Pavoine & Bonsall 2011). Here, we re-examine this issue based on a consistent empirical data set. We integrate an extensive trait data set and a species-level phylogenetic tree to test assembly scenarios in tropical tree communities of French Guiana, South America. We measured 17 functional traits for 4672 trees of 668 species in nine 1-ha permanent plots, and we developed a project-specific phylogenetic hypothesis for the same species estimated from sequences of the *rbcL* and *matK* genes. We used this unique data set to address the following questions: (i) Under which community assembly process is the structure of local communities consistent? (ii) Are tests of niche overlap consistent between trait-based and phylogeny-based approaches? We addressed (i) by testing whether locally co-occurring species were more functionally and phylogenetically clustered than expected by chance alone and (ii) by conducting a comprehensive analysis of the extent to which functional traits were phylogenetically correlated in this community. We discuss the results and implications for the use of trait and phylogenetic approaches in community ecology.

Materials and methods

FIELD SAMPLING AND BOTANICAL IDENTIFICATION

Field sampling was conducted in 2007 and 2008 at nine 1-ha plots representing a gradient of precipitation and geological substrates across lowland tropical forests in French Guiana (for sampling site characteristics, see Appendix S1 in Supporting Information). In each plot, all trees > 10 cm diameter at breast height (d.b.h.) were mapped and measured for height and d.b.h. Each tree was sampled by tree climbers to obtain leaf and twig tissues.

Herbarium vouchers were collected for every stem to verify botanical identifications at Herbier IRD de Guyane (CAY), with consultation of taxonomic specialists as necessary. A number of taxonomic issues in identification were resolved using DNA barcoding (Gonzalez *et al.* 2009). Overall, 4672 stems were sampled belonging to 668 taxa: 499 valid species (encompassing 4348 individuals) and 169 morphospecies (324 individuals, or 6.9% of the total). These taxa represented a total of 221 genera and 58 families (*sensu* Angiosperm Phylogeny Group III, 2009).

FUNCTIONAL TRAIT MEASUREMENT

For each tree, 17 leaf and trunk functional traits were measured (Table 1, Baraloto *et al.* 2010a). In the final data set, some of the traits were log-transformed to improve normality. On the basis of these trait values, we defined two major axes of variation in the trait data via a principal component analysis. The loadings along the two first axes defined two compound trait variables, henceforth referred to as PCA1, and PCA2. These axes were interpreted as axes of leaf-trait (leaf economics spectrum, Wright *et al.* 2004) and stem trait variation (stem economics spectrum, Baraloto *et al.* 2010b), which are thought to more completely approximate the niche occupancy of the species than individually selected traits. More details on the construction of these variables are available in Baraloto *et al.* (2010b).

MOLECULAR ANALYSES AND PHYLOGENY RECONSTRUCTION

Leaf tissue was collected for each tree sampled in the field and immediately stored in silica gel. Up to 30 mg of dry material was ground for 2 min in a TissueLyser mixer-mill disruptor (Qiagen, Valencia, CA, USA) using tungsten beads. Lysis incubation was carried out at 65 °C over 2 h for cambium tissue and 1 h for leaf tissue using cetyl trimethylammonium bromide 1% polyvinyl pyrrolidone buffer. Total DNA extraction was performed using a Biosprint 15 workstation (Qiagen) following the manufacturer's protocols. For PCR amplification and sequencing of the *rbcL* exon, primer combinations were 1f (5′ ATGTCACCACAAACAGAAAC) and 1460r (5′ TCCTTTTAGTAAAAGATTGGGCCGAG; Chase *et al.* 1993) with internal primers 724r (5′ TCGCATATGTACCTGCAGTAGC) and 636f (5′ GCGTTGGAGAGATCGTTTCT). For *matK*, a combination of order-specific primers was used as described in Dunning & Savolainen (2010). All PCRs contained 20 μL of 1.1× ReddyMix PCR Master Mix (Thermo Fisher Scientific Inc., ABGene, Epsom, UK), 0.2 μL of GoTaq® 5U μL^{-1} (Promega, Madison, WI, USA), 0.02 μmol L^{-1} each of the forward and reverse primers and 20–50 ng of template DNA. In addition, for *matK*, dimethyl sulfhoxide (Sigma, St Quentin Fallavier, France) was added to make 4% of the total volume. The *matK* PCR profile used: 94 °C for 5 min, 38 cycles of 94 °C 30 s, 48 °C for 40 s, 72 °C for 40 s, with a final extension of 72 °C for 7 min. *rbcL* PCR profile used: 94 °C for 5 min, 34 cycles of 94 °C for 1 min, 48 °C for 40 s, 72 °C for 90 s, with a final extension of 72 °C for 7 min. PCRs were performed on Applied Biosystems (Foster City, CA, USA) GeneAmp PCR machines. Sequencing reactions had a total volume of 10 μL and contained: 0.5 μL BigDye® Terminator v3.1 (Applied Biosystems), 3 μL of 2.5× Sequencing Buffer (Applied Biosystems), 0.02 μmol L^{-1} of forward or reverse primers and *c*. 35 ng of purified PCR product. The cycle sequencing used the following profile: 94 °C for 1 min, 25 cycles of 96 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min. A total of 606 *rbcL* sequences (1320 bp) and 244 *matK* sequences (891 bp) were obtained, with at least one sequence per genus for *matK*. These sequences were deposited in GenBank/EBI (accession numbers JQ625717-JQ626579).

To construct a dated phylogenetic hypothesis, we constructed a tree using maximum likelihood with RAXML v7.0.1 software (Stamatakis, Hoover & Rougemont 2008) and with two independent partitions for the two plastid regions, via the CIPRES on-line portal (Miller *et al.* 2010). We then implemented the nonparametric rate smoothing method of Sanderson (1997) to make this tree ultrametric. This preliminarily smoothed tree was used as the starting tree in a simultaneous estimation of phylogenetic topology and divergence times with

Table 1. Functional traits measured in the study, along with the units, number of individuals measured for each trait, the median and range of observed species mean trait values, and the mean within-species trait variation

						Functional clustering				Phylogenetic signal (K)	
Functional traits	Units	N	Median	Range in data set	Intraspecific variation	U_{ST}	SES	τ_{ST}	SES	Without intraspecific variation	With intraspecific variation
Foliar $\delta^{13}C$ composition	‰	2946	−32.07	−36.44 to −25.67	0.553	0.0229	5.43**	0.0134	9.75**	0.051	0.214
Foliar C:N ratio	g g^{-1}	2947	24.01	7.79–59.77	0.274	0.0242	5.52**	0.0118	9.36**	0.071	0.134
Foliar K concentration	%	933	0.05	0.114–2.235	0.548	0.0155	5.05**	0.0233	19.86**	0.072	0.202
Foliar N concentration	%	2948	2	0.762–6.190	0.273	0.0211	6.57**	0.0213	15.32**	0.077	0.160
Foliar P concentration	%	933	0.1	0.024–0.251	0.447	−0.0022	−0.55	0.0017	1.4	0.075	0.212
Laminar chlorophyll content	μg mm^{-2}	4611	70.5	10.3–255.1	0.551	0.0260	6.47**	0.0083	7.12**	0.043	0.147
Laminar total surface area	cm^2	4587	72.1	2.032–643 700	0.064	0.0212	6.14**	0.0087	7.99**	0.143	0.207
Laminar toughness	N	4590	1.65	0.22–13.06	0.256	0.0050	1.35	0.0045	3.70**	0.051	0.109
Leaf tissue density	g cm^{-3}	4540	0.042	0.008–0.287	0.53	0.0208	5.96**	0.0227	15.18**	0.047	0.123
Leaflet surface area	cm^2	4587	52.23	0.018–3218	0.182	0.0055	1.26	0.0033	2.88**	0.088	0.131
NH_4^+ utilization	%	4587	47	1–100	0.452	0.0045	1.14	0.0054	4.44**	0.057	0.162
Specific leaf area	cm^2 g^{-1}	4577	10.68	1.77–47.41	0.456	0.0106	2.41*	0.0013	1.24	0.038	0.088
Trunk bark thickness†	mm	3805	4	0.0–53.0	0.692	0.0195	4.42**	0.0118	10.52**	0.050	0.323
Trunk xylem density†	g cm^{-3}	2844	0.65	0.23–0.98	0.252	0.0235	6.11**	0.0051	6.13**	0.100	0.216
Trunk xylem moisture content†	%	2256	61.4	17–287	0.451	0.0015	0.36	0.0089	7.41**	0.101	0.318
Twig bark thickness†	mm	2369	1.95	0.07–7.31	0.563	0.0173	4.35**	0.0224	17.64**	0.043	0.179
Twig xylem density†	g cm^{-3}	2390	0.616	0.19–0.96	0.34	0.0192	5.10**	0.0113	8.72**	0.076	0.201
PCA 1 (Leaf economics)					0.467	0.0296	7.76**	0.0262	19.28**	0.047	0.150
PCA 2 (Stem economics)					0.23	0.0150	3.53**	0.0047	4.19**	0.085	0.147

U_{ST} and τ_{ST} express the individual-based and species-based trait structure statistics, respectively. Significance was tested by randomizing the species (see Fig. 1). For both metrics, we reported the standard effect size (SES: mean effect divided by the standard deviation of the null distribution), and the one-tailed P-value. The P-value tests the null model where species are permuted randomly among themselves in the matrix of species traits. Trait phylogenetic signal was measured using Blomberg's K using species mean trait values, and accounting for intraspecific trait variation. All K values departed significantly from the null model of no phylogenetic signal ($P < 0.01$).
*$P < 0.05$; **$P < 0.0001$.
†Log-transformed prior to analysis.

a Bayesian MCMC approach (BEAST v1.5.3; Drummond & Rambaut 2007). We implemented an uncorrelated relaxed molecular clock, which draws substitution rates from a lognormal distribution independently for each branch in the phylogenetic tree. To temporally calibrate the phylogenetic tree, we placed constraints on divergence times for minimum crown age at nine nodes spread across the phylogeny (Appendix S2). This choice spans the angiosperms, particularly concentrating on groups most represented in our data set. We placed a lognormal prior on node age, with hard minimal constraints corresponding to 90% of the estimated fossil date. After preliminary runs of 1–2 million generations to optimize the MCMC exploration of parameter space, we implemented two MCMC chains of 30 million generations, sampling trees and parameter values every 10 000 generations. After confirming convergence of the two runs, we combined the post-burn-in samples of the two runs (burn-in of 15 million generations). We generated a maximum clade credibility tree from this set, with node ages represented as the mean across all trees in the posterior distribution. The inferred age of the root was close to 200 million

years, a result consistent with recent analyses (Smith, Beaulieu & Donoghue 2010), although older than that traditionally recognized.

TESTING COMMUNITY-WIDE TRAIT STRUCTURE

Community-wide structure tests explore whether a local sample of the community consists of a random assemblage of the species found in samples from a larger region (sometimes referred to as the metacommunity). Departures from this null expectation signal ecological processes that shape community assembly, such as competitive exclusion or ecological filtering.

We first addressed whether co-occurring species had more similar or dissimilar traits than expected by chance. We calculated the mean Euclidean distance in trait space for two individuals of distinct species picked at random (i) from within the same plot (D_w^{*T}) and (ii) from different plots (D_a^{*T}). These indices are reminiscent of Rao's diversity index, defined as the mean functional distance between individuals (Hardy & Senterre 2007; Hardy 2008; Eastman, Paine & Hardy 2011). By analogy with similar spatial structure statistics in population genetics, community-wide trait structure was measured as $U_{\mathrm{ST}} = 1 - D_w^{*T}/D_a^{*T}$. Unlike Wright's fixation index (F_{ST}), U_{ST} may take negative values. Trait overdispersion is observed if $U_{\mathrm{ST}} < 0$, that is, if two individuals of distinct species are more dissimilar within a site than in two distinct sample sites, and trait clustering is indicated if $U_{\mathrm{ST}} > 0$. The latter can be interpreted as a functional turnover among sites. Because the indices D_w^{*T} and D_a^{*T} are constructed from pairs of individuals in distinct species, $U_{\mathrm{ST}} > 0$ cannot be due to taxonomic similarity among plots, but must be explained by trait similarity.

A related metric called τ_{ST} is defined similarly but ignores species abundances. Defining Δ_w^T as the mean trait distance between distinct species within a plot and Δ_a^T as the mean trait distance for distinct species picked from different plots $\tau_{\mathrm{ST}} = 1 - \Delta_w^T/\Delta_a^T$. The advantage of these indices is that they provide a comparable metric for single traits, for ensembles of traits and for PCA loadings. Consistent results for both U_{ST} and τ_{ST} ensure that results are not biased by a possible correlation between trait values and species abundances.

Significance of the comparison between U_{ST} or τ_{ST} and zero was obtained by comparing the observed statistic to 300 randomizations of the species labels in the data set following a procedure described in Hardy & Senterre (2007), and called randomization scheme 1a in Hardy (2008).

TESTING COMMUNITY-WIDE PHYLOGENETIC STRUCTURE

We used the same approach to construct phylogenetic community structure indices, defined as follows: D_w^{*P} and D_a^{*P} were the mean divergence time between individuals of distinct species sampled within a plot, or in different plots, respectively. Then, abundance-based community-wide phylogenetic structure was measured as $B_{\mathrm{ST}} = 1 - D_w^{*P}/D_a^{*P}$. Occurrence-based community structure was computed from Δ_w^P and Δ_a^P, the mean phylogenetic distance between distinct species sampled within a plot and from different plots, respectively: $\Pi_{ST} = 1 - \Delta_w^P/\Delta_a^P$. For both indices, significantly positive values are indicative of local phylogenetic clustering (Hardy & Senterre 2007) and can be interpreted as a phylogenetic turnover among sites. As above, consistent results for both B_{ST} and Π_{ST} ensure that results are not biased by a possible phylogenetic signal in species abundances.

Significance of the comparison between B_{ST} or Π_{ST} and zero was obtained by comparing the observed statistic to 300 randomizations following scheme 1a as in Hardy (2008).

ESTIMATING AND TESTING FOR PHYLOGENETIC SIGNAL

We used Blomberg's K statistic to assay for phylogenetic signal, the tendency of related species to resemble one another (Blomberg, Garland & Ives 2003; Losos 2008). If there is no phylogenetic signal, K will be close to zero. Under a Brownian motion model, where traits change by small random amounts and at a constant rate through time, K is expected to be equal to one. Under phylogenetic trait conservatism (sensu Losos 2008), where lineages diverge early in trait values but are subsequently restricted to a limited range of trait values, K may be greater than one. A hypothetical example of phylogenetic trait conservatism would be if every taxonomic family shows a different value for a given trait, but all members of a given family have the same trait value. Alternatively, divergent selection among closely related species or convergent evolution in distant lineages would reduce K values. In this way, different evolutionary processes or combinations of processes can result in the same value of K (Revell *et al.* 2008). Our objective was to estimate the amount of phylogenetic signal, not to disentangle the processes responsible. We therefore generated a null expectation of K under no phylogenetic signal by randomly shuffling the tips of the phylogeny 1000 times. Significant phylogenetic signal is indicated if the observed K value is greater than across 95% of the randomizations.

Using a single trait measurement per species can underestimate phylogenetic signal if the trait has been measured with error and/or if there is intraspecific variation for the trait (Blomberg, Garland & Ives 2003; Ives, Midford & Garland 2007). Although measuring multiple individuals and using the mean value should improve estimates of phylogenetic signal, a more accurate estimate can be obtained if the standard error of trait measurements for each species is also incorporated into the analysis (Ives, Midford & Garland 2007). We implemented the approach of Ives, Midford & Garland (2007) to estimate Blomberg's K while incorporating intraspecific variation (intraspecific standard error). For species that had been measured only once for a given trait, the intraspecific standard error was estimated as the mean of the standard deviation of all species that had multiple measurements (*sensu* Ives, Midford & Garland 2007). We compared these estimates of K to conventional estimates of K in which only mean values were used and standard errors were not incorporated. Additionally, we quantified the magnitude of variation within species for each trait as the residual variation in trait measurements not explained by species identity (i.e. 1 − the adjusted R^2 of a linear model with species as the explanatory term). This intraspecific measurement variation could represent measurement error and/or real intraspecific variation, and our data set does not permit us to distinguish between the two.

To visualize how trait similarity varied with phylogenetic depth, we constructed phylogenetic autocorrelograms for each trait and the first two PCA components. We measured similarity between two species, i and j, for a given trait as $I_{ij} = \frac{(x_i - \bar{x})(x_j - \bar{x})}{V_x} + 1/(n-1)$, where x_i is the mean trait value for species i, $\bar{x}$ and V_x are the mean and variance, respectively, of the mean trait values across all species and n is the number of sampled species. The second term is a sampling bias correction ensuring that the mean of I_{ij} over species pairs equals zero. The phylogenetic autocorrelograms plotted average values of I_{ij} for a set of contiguous divergence time intervals δ_{ij}, using bin widths of 20 Myr. We also plotted the mean and 95% confidence intervals

under no phylogenetic signal. This null expectation was generated by re-calculating the mean I_{ij} values one thousand times on randomized tips of the phylogeny.

To test for the presence of phylogenetic trait conservatism on I_{ij}, we simulated the Brownian evolution of each trait and of their ordination loadings along the phylogeny. After simulated trait values were obtained for all tips of the phylogenetic tree, they were normalized while accounting for intraspecific variation (by the addition of a random error term following a centred normal distribution with SD = standard errors of normalized mean species trait values) so that the variance in simulated trait values equals V_x as defined above and intraspecific variation contributes to V_x in the same proportion as the real data. One thousand independent simulations were used to obtain the 95% envelope of average I_{ij} per time interval for each trait and PCA components.

Statistical analyses were conducted in the R 2.11.1 statistical platform (R Development Core Team), using the packages SPACODIR v.0.11 (Eastman, Paine & Hardy 2011), phytools (Revell 2011) and ape (Paradis, Claude & Strimmer 2004), and by using SPACODI0.10 (Hardy 2010).

Results

COMMUNITY-WIDE TRAIT STRUCTURE

The tropical tree communities of French Guiana displayed significant phenotypic clustering when the phenotypic distance was computed across all 17 traits (Fig. 1a,b) and for the two first PCA loadings (Fig. 1c,d). Hence, local communities contained species or individuals with more similar traits than expected by chance. To test for the consistency of our results at smaller spatial scales, we replicated this analysis by considering 0.25-ha subplots as the sampling units (n = 36) and found the same results that species or individuals in subplots had more similar traits than expected by chance (not shown).

We also studied the same pattern trait by trait. Of the 17 traits, 13 and 15 displayed significant spatial clustering according to U_{ST} and τ_{ST}, respectively (Table 1). Only NH_4 uptake and total leaf area displayed no spatial trait clustering. For the two first PCA loadings taken separately, significant functional clustering was also observed (Table 1), but the signal was stronger for the first axis (U_{ST} = 0.0296), representing the leaf economic spectrum, than for the second (U_{ST} = 0.0150), representing the stem economic spectrum. The same pattern held when species abundance was ignored (τ_{ST} = 0.0262 for the first PCA loading; τ_{ST} = 0.0047 for the second PCA loading).

PHYLOGENETIC COMMUNITY STRUCTURE

Significant phylogenetic clustering was observed using both indices based on species abundances (B_{ST} = 0.0126, Fig. 2a) and based on species occurrences (Π_{ST} = 0.0030, Fig. 2b). The result also held when we considered 0.25-ha subplots as the sampling units (n = 36, results not shown). We also assessed the effect of phylogeny reconstruction on this signal

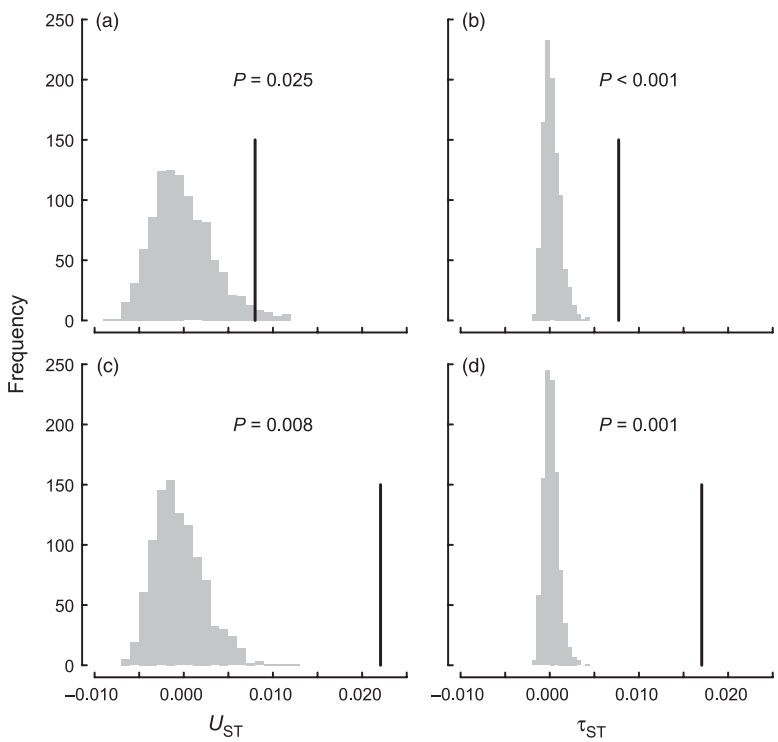

Fig. 1. Community-wide trait structure as measured based on all 17 functional traits (panels a and b) or on the two PCA loadings (panels c and d), and both abundance-based metrics (U_{ST}, panels a and c) and abundance-independent metrics (τ_{ST}, panels b and d). In all four cases, 1000 randomizations were performed to construct the null distribution (grey histogram), compared with the observed value (black vertical bar). Co-occurring individuals and species had more similar traits than expected by chance. The observed values were as follows: (a) 0.008, (b) 0.0077, (c) 0.0221, (d) 0.0171.

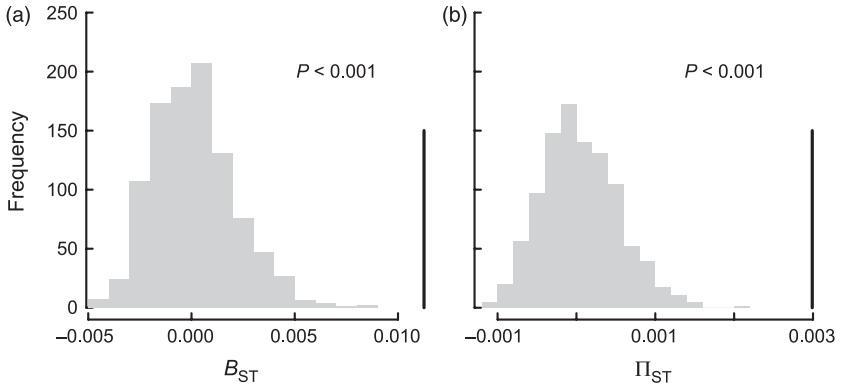

Fig. 2. Community-wide phylogenetic structure as measured based on both abundance-based metrics (B_{ST}, panel a) and abundance-independent metrics (Π_{ST}, panel b). In both panels, 1000 randomizations were performed to construct the null distribution (grey histogram), compared with the observed value (black vertical bar). Co-occurring individuals and species were more phylogenetically similar than expected by chance.

by conducting the above analysis on 100 trees selected at random from the post-burn-in MCMC search of the tree space. Our conclusions regarding the significance of phylogenetic clustering were robust to variation in tree topology.

The phylogenetic tree produced here agrees well with that proposed by the Angiosperm Phylogeny Group III (2009) (Appendix S2). Nevertheless, we also tested the robustness of our approach with respect to phylogenetic resolution using a phylogenetic tree derived from the angiosperm family-level tree of Davies *et al.* (2004), where we then added French Guianan genera and species as polytomies within their respective families (Angiosperm Phylogeny Group III 2009). Significant phylogenetic clustering was still detected and values were similar (B_{ST} = 0.0084, Π_{ST} = 0.0023), indicating that most of the signal detected in the main analysis was caused by deeper splits within the phylogeny.

The four indices measuring community structure, two abundance-based indices U_{ST}, B_{ST} and two occurrence-based indices τ_{ST}, Π_{ST}, are normalized and constructed similarly. As a result, the intensity of trait community structure and phylogenetic community structure may be compared. Trait community clustering, especially based on the first two components of the PCA (Fig. 1c,d), was more intense than phylogenetic clustering (Fig. 2), both for abundance-based (i.e. $U_{ST} > B_{ST}$) and for occurrence-based comparisons (i.e. $\tau_{ST} > \Pi_{ST}$).

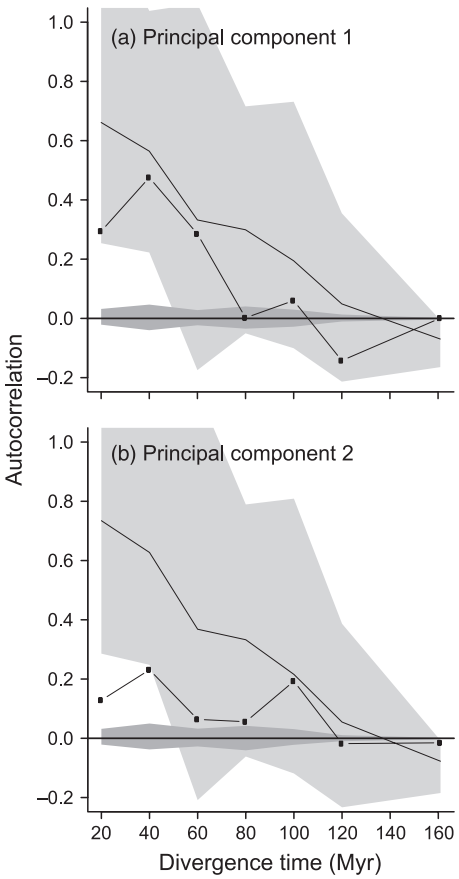

Fig. 3. Phylogenetic autocorrelograms of the first (panel a) and second (panel b) PCA loadings of traits (connected squares). Black line and light grey area: expected and 95% envelopes under the null hypothesis of a Brownian evolution model. Dark grey area: 95% envelopes under the null hypothesis of no phylogenetic signal.

PHYLOGENETIC SIGNAL AND CONSERVATISM

All traits showed a significant phylogenetic signal over the entire taxonomic range considered here, both in analyses accounting for intraspecific measurement variation and when only the mean trait values were used (Table 1). Likewise, the first two PCA components displayed significant phylogenetic signal in both cases (Table 1). However, the estimates of K were substantially lower when intraspecific measurement variation was ignored. In fact, when K was calculated using just mean trait values, the estimates of K for individual traits are significantly negatively related to their magnitude of intraspecific measurement variation (linear model: adjusted R^2 = 0.49, $P < 0.001$). This relationship disappeared when intraspecific measurement variation was accounted for when estimating K (linear model: adjusted R^2 = 0.05, P = 0.35).

The phylogenetic autocorrelograms show how trait similarity varies with depth in the phylogenetic tree (see Fig. 3 for PCA components 1 and 2 and Appendix S3 for all traits). In general, traits show declining similarity at increasing depths

and higher similarity in recent divergence classes (i.e. among close relatives) than expected under the 'no phylogenetic signal' scenario. This is commensurate with our finding of significant phylogenetic signal. Closely related species were less similar than expected under Brownian motion evolution for eight traits and the second PCA component according to the I_{ij} statistic (Fig. 3, Appendix S3). Additionally, all traits and the first two principal components showed K values that were lower than would be expected under Brownian motion evolution (Table 1). No trait or PCA component showed a pattern indicative of phylogenetic conservatism.

Finally, we found no apparent relationship between the amount of phylogenetic signal displayed by individual traits (Blomberg's K) and their pattern of clustering within communities (results not shown).

Discussion

We performed a comprehensive test of the hypothesis that tropical tree communities in French Guianan rain forests are randomly assembled. We offer evidence against this hypothesis by combining a detailed analysis of both functional and phylogenetic community structure. We report two major results. First, similar species are clustered in local communities, in terms of functional trait similarity and, to a lesser extent, phylogenetic similarity (Figs 1 and 2). In other words, both functional and phylogenetic turnover occur among local communities. Second, the traits we measured all show significant phylogenetic signal (Table 1, Fig. 3), further supporting the assumption that phylogenetic proximity between species pairs may be used as a proxy of niche overlap (Webb *et al*. 2002). These results are inconsistent with those reported for Barro Colorado Island, Panama, by Swenson & Enquist (2009), the only other study of tropical forests we know of that integrated both phylogenetic and trait community structure analyses. These differences may be due to the way data were analysed, or to differences in phylogenetic and spatial scales (Cavender-Bares, Keen & Miles 2006; Cavender-Bares *et al*. 2009; Swenson & Enquist 2009; Kraft *et al*. 2010) as well as the scale and resolution at which functional trait measures were made. Later, we interpret our results in the light of these differences in scale; and we then discuss the importance of phylogenetic signal for interpreting patterns of trait and phylogenetic structure in this and other studies.

BIOGEOGRAPHIC SCALE

Biogeographic scale is important to analyses of community assembly in at least three ways: relative to dispersal limitation, the definition of a regional species pool and the influence of environmental gradients across which local communities are distributed.

Previous tests were performed with respect to a null hypothesis assuming a fully randomized data set (Cavender-Bares *et al*. 2004; Vamosi *et al*. 2009), which makes the assumption that all species can disperse everywhere. However, if dispersal is limited and if dispersal ability shows a phylogenetic signal, then the assumption of panmixis breaks down. There is substantial evidence for dispersal limitation in tropical forest trees (Condit *et al*. 2002; Pennington, Richardson & Lavin 2006). To account for this potential issue, the randomization scheme chosen here maintained the spatial autocorrelation in local species abundances (Hardy 2008). However, the correlation of spatial patterns between species pairs does break down when ranking species pairs according to phylogenetic proximity. In addition, the tests of community structure carried out here are based on inter-specific comparisons. Therefore, even if dispersal limitation causes a spatial pattern in local species abundances, dispersal limitation does not bias the test as long as it does not cause a spatial correlation between the local abundances of related species. The latter pattern would occur if speciation rate is negligible compared to dispersal rate. Given the biology of tropical tree species, we do not expect to observe such a pattern at our study scale (see Hardy *et al*. 2012, for a similar analysis at the intercontinental scale).

A second issue of biogeographic scale is to what extent the species sampling is representative of the regional species pool. Simulation-based analyses have revealed that studies on community-wide phylogenetic structure representing 30–60% of the regional diversity have the greatest statistical power to detect phylogenetic community structure (Kraft *et al*. 2007). The present analysis includes more than 40% of the tree species diversity of French Guiana (in excess of 1600 species) within the suitable range to detect patterns. Hence, our finding of a phylogenetic clustering is robust at least to the sampling intensity of the regional species pool. Nevertheless, more robust methods framing phylogenies in a spatially explicit setting may be useful to reassess our results (Freckleton & Jetz 2009). Also, ongoing efforts to increase phylogenetic and trait sampling in French Guiana and other tropical forests will certainly improve the resolution of these analyses.

A third related issue involves the environmental gradient across which local communities are sampled. Environmental filtering may be particularly strong when examining local communities differing in topographic position (Kraft, Valencia & Ackerly 2008) or across strong edaphic or climatic gradients (Cavender-Bares, Keen & Miles 2006; Fine & Kembel 2011). Our study covered broad climatic gradients at a scale of ca. 20 000 km^2 but we limited sampling to forests on terra firme clay-rich sediments (Table 1). As such, our results may have underestimated the strength of environmental filtering across broader edaphic gradients existing in the region (Hammond 2005; Hoorn & Wessenlignh 2010). In the light of climate change scenarios predicted for the region, further investigations would yield useful insights, particularly by integrating the dry forests of the interior of the Guiana Shield, which have a unique floristic composition (Clarke & Funk 2005; ter Steege *et al*. 2006).

PHYLOGENETIC SCALE AND RESOLUTION

Phylogenetic scale is also important to the interpretation of studies in at least two ways – the resolution of the available data and the scale of focus (a single clade vs. a local community).

Many of the recent tests of community-wide phylogenetic signal in plants have relied on Davies *et al.* (2004)'s phylogenetic tree, which used a supertree approach for completeness at family level (Bininda-Emonds, Gittleman & Steel 2002), and for which branch length computation was based on optimizing *rbcL* onto the topology. Additionally, Davies *et al.* (2004) dated the tree using Sanderson (1997)'s nonparametric rate smoothing method and a single calibration point (the Fagales-Cucurbitales split). Here, we generated DNA sequences for the same plants for which traits were measured, including morphospecies. We then used these sequences to generate a dated phylogenetic hypothesis by Bayesian inference with nine calibration points (Appendix S2). This procedure allowed us to reduce the uncertainty in the assignment of morphospecies to clades and to provide a higher level of taxonomic confidence to our study.

The full control over both traits and phylogeny was an advantage when controlling for possible error propagation issues. For instance, we were able to assess the effect of phylogeny reconstruction on the phylogenetic community structure test by conducting the same analysis on 100 trees selected at random from the post-burn-in MCMC search of tree posterior probability space. Our conclusions were robust to variation in tree topology. Qualitatively, they were even robust to the use of a phylogenetic tree essentially unresolved within families.

FUNCTIONAL TRAIT RESOLUTION

If traits relevant to species' niches are available, why use phylogenetic similarity as a proxy for niche overlap at the community scale? Indeed, the phylogeny sub-tending co-occurring species may be too coarse to serve as a proxy for species niche overlap (Cavender-Bares *et al.* 2009). Nevertheless, using traits to delineate the niches of species is not without problems either; relevant functional traits may not be measurable for all or a subset of the species (especially rare ones), or may simply be overlooked (Baraloto *et al.* 2010a). For example, some Neotropical trees are Al-accumulators, and this trait probably has adaptive significance, even though it is not considered in most trait studies (Jansen *et al.* 2002). Our study represents one of the most comprehensive approaches for a functional trait-based definition of plant species niches to date, yet we did not include several potentially important axes of trait variation. As collaborations to compile trait data bases increase (Kattge *et al.* 2011), an integration of data on life history and reproduction ecology is needed (ter Steege *et al.* 2006) in addition to metrics of plant chemical defences such as tannins and phenolics for which rapid assays are now available (Cerovic *et al.* 2008).

Integrating trait measures at a community level in species-rich systems may also be difficult because intraspecific variation in trait values can be substantial (Baraloto *et al.* 2010a; Albert *et al.* 2012). There has been a great deal of recent research to understand the role of intraspecific trait variation both in comparative methods in evolution (Blomberg, Garland & Ives 2003; Ives, Midford & Garland 2007) and to understand the functioning of ecological processes (Clark 2010; Lavergne *et al.* 2010; Sandel *et al.* 2010). However, the potential contribution of intraspecific trait variability on community-wide trait signal has seldom been studied (Messier, McGill & Lechowicz 2010; Albert *et al.* 2012). Indeed, an analysis of trait clustering in the same plots reported here found that the strength of environmental filtering increased markedly when individual-level measures of functional traits were used rather than species means (Paine *et al.* 2011). Thus, the choice of traits, the measure of their variation within species and the extent to which phylogenetic information may be used as a surrogate for trait similarity all merit further attention.

PHYLOGENETIC SIGNAL OF FUNCTIONAL TRAITS

All of the traits we measured, be they characteristics of leaf structure, leaf chemistry, or stems, showed significant phylogenetic signal (Table 1). This suggests that phylogenetic relatedness, at the scale of the entire tree, can serve as a reasonable proxy for trait similarity. Furthermore, both trait and phylogenetic community signal were consistent with an interpretation of environmental filtering. These results are indicative that phylogenetic proximity may be a surrogate for niche overlap (Cavender-Bares *et al.* 2004; Vamosi *et al.* 2009; Pausas & Verdú 2010). In tropical rain forest tree communities, a consistent phylogenetic signal for climate niche has been shown to occur along regional rainfall gradients and to be well correlated among continents (Hardy *et al.* 2012), indicating that initial adaptations for particular climatic conditions tend to be well conserved within at least some major angiosperm clades.

Visual inspection of the phylogenetic autocorrelograms shows that mean trait similarity in the most recent divergence class (0–20 Myr) is often less than in the next deepest divergence class (20–40 Myr) (Fig. 3, Appendix S3). This could suggest that there has been divergent selection among the most closely related species or convergent evolution across lineages. This is also the probable reason for our low estimates of *K*. Nonetheless, when we look across all phylogenetic depths, species in the more recent divergence classes show greater trait similarity than those in the deepest divergence classes (Fig. 3, Appendix S3). Finally, trait similarity was below the Brownian motion envelopes in Fig. 3 and Appendix S3, and the *K* estimates were lower than 1, which suggests that there is no phylogenetic conservatism in the traits and their ordination loadings (Losos 2008).

As pointed out repeatedly, phylogenetic signal or conservatism of traits should not be assumed *a priori*, and instead should be tested explicitly. In testing these assumptions, however, considerable confusion has been generated by the choice of terms because the community phylogenetics literature frequently invokes 'phylogenetic conservatism' to mean phylogenetic signal rather than phylogenetic conservatism sensu Losos (2008). As found in the present study, functional traits might not be phylogenetically conserved, in reference to a Brownian motion model of evolution, but still show phylogenetic signal insofar as trait similarity is related to phylogenetic similarity.

Recent analyses have suggested that intraspecific trait variability, due to measurement error and/or real intraspecific

variation, can lead to underestimation of phylogenetic signal for traits (Ives, Midford & Garland 2007; Felsenstein 2008; Freckleton & Jetz 2009). Our analyses support this contention (Table 1). Furthermore, we found that when K is calculated by the conventional method, using just the mean of trait values, the estimated K seems largely dependent on the magnitude of intraspecific measurement variation. Thus, we advocate incorporating measurement variation within species, when available, to avoid bias when assessing phylogenetic signal for traits (*sensu* Ives, Midford & Garland 2007).

Conclusion

This study provides further evidence that communities are not randomly assembled, and instead, that niche-based processes appear to have a stabilizing effect on biodiversity at the regional scale in megadiverse species assemblages. We quantified the magnitude of this signal using unbiased metrics that can be compared across communities. In our study, phylogenetic clustering was found to be less intense than functional clustering, suggesting that when sufficiently broad trait diversity can be assayed, trait-based approaches are a powerful alternative to more easily implemented phylogeny-based approaches. However, our results – as most recently published comparable results – may be highly contingent on the choice of the set of local communities, and on the selection of functional traits. Larger-scale studies and long-term projects in functional ecology would be needed to address both issues.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Sampling site characteristics.

Appendix S2. Phylogenetic tree reconstruction.

Appendix S3. Phylogenetic trait signal and conservatism.

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