FUNCTIONAL LEAF TRAIT DIVERSITY OF 10 TREE SPECIES IN CONGOLESE SECONDARY TROPICAL FOREST

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VERBEECK H, BETEHNDOH E, MAES WH, HUBAU W, KEARSLEY E, BUGGENHOUT L, HUFKENS K, HUYGENS D, VAN ACKER J, BEECKMAN H, MWERU JPM, BOECKX P & STEPPE K. 2014. Functional leaf trait diversity of 10 tree species in Congolese secondary tropical forest. The Congo Basin has a large secondary forest area. Nevertheless, global plant trait databases lack substantial data from this biome and functional trait diversity is largely unknown. We analysed a unique leaf trait dataset (specific leaf area, nutrient and isotope concentrations) collected from 88 individual trees belonging to 10 different species in tropical lowland forest in the Democratic Republic of the Congo. The trait data were found to be consistent with global datasets. $\delta^{15}N$ was the only trait significantly influenced by plot location. For all other leaf traits, shade tolerance was a significant factor. The species factor was significant within each shade tolerance class. This shows that shade tolerance is an important but not exclusive factor determining functional diversity. Tree height had significant influence on $\delta^{13}C$, specific leaf area and area-based nutrient concentrations. Higher individual trees had thicker sun-adapted leaves, regardless of the species. A principal component analysis (PCA) resulted in three significant ordination axes: leaf-thickness, N-content and P-content. By hierarchical clustering of the PCA scores, four functional groups were distinguished. This showed that species with diverse strategies coexisted in the ecosystem.

Keywords: Isotopes, nitrogen, specific leaf area, phosphorus, wood density

INTRODUCTION

The Congo Basin forest is the world's second largest continuous block of tropical rainforest and has relatively modest deforestation rates compared with the Amazon and South-East Asia (De Wasseige et al. 2010). Yet, forest conversion in the Congo Basin is substantial and mainly driven by shifting cultivation, resulting in a relatively large secondary forest area compared with other continents (FAO 2011).

Despite its importance for the global carbon cycle and biodiversity, the Congo Basin rainforest biome is largely overlooked in most global studies (Verbeeck et al. 2011). Compared with the Amazon Basin, field studies on the carbon balance, physiology and ecology of Congo's forests remain very limited (Ebuy et al. 2011). As

poor logistics limit the possibilities for detailed ecophysiological or biogeochemical process monitoring and manipulation experiments, the collection of plant traits can offer better insight into the functioning of Congo's forests. Plant traits are morphological, anatomical, biochemical, physiological or phenological features measurable at the individual level (Violle et al. 2007). These traits can reflect the response of plants to abiotic and biotic constraints (Kattge et al. 2011) and their variability represents functional diversity in ecosystems. In addition, functional traits can be used to characterise the functional divergence of different coexisting species within an ecosystem. This can possibly help to understand how such a large amount

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of species can coexist in highly diverse tropical forest ecosystems.

Unfortunately, global plant trait databases lack substantial data from the Congo Basin (Kattge et al. 2011). As an attempt to contribute to the urgently needed gap filling of leaf trait data for the Congo Basin, this paper presents a limited but unique dataset of leaf traits collected from 88 individual trees of 10 different species in seven plots of secondary tropical lowland forest in three forest reserves in the region of Kisangani, Democratic Republic of the Congo.

The first objective of this study was to characterise the leaf trait variability between and within species and plot locations. We hypothesised that tree species with different functionalities coexisted within these secondary tropical forests. In other words, we tested if the niche theory (e.g. Sterck et al. 2011) was applicable on these ecosystems, which were young and appeared relatively homogeneous at first sight. Based on the observed leaf traits, we tried to determine distinct functional species groups.

A second objective of this study was to establish general relations between different leaf traits and tree biometry. Such relations are valid for all functional groups within these ecosystems and are determined by resource limitations (i.e. light, water or nutrients). We hypothesised that some general correlations existed between different leaf traits on the one hand, and between leaf traits, tree height and/or wood density on the other.

MATERIALS AND METHODS

Study sites

Leaf samples were collected in July and August 2010 from three forest reserves in the region of Kisangani in north-eastern Democratic Republic of the Congo. This region has tropical rainforest climate (Af type; Peel et al. 2007). Temperature is relatively constant (25.2 °C) and rainfall is high throughout the year (more than 1800 mm year^{-1}). Two short dry seasons occur here: December–February and June–August (Ebuy et al. 2011). The Yoko Reserve (YO) is located between 21 and 34 km south of Kisangani. The UNESCO Man and Biosphere Reserve of Yangambi (YA) is located about 100 km west of Kisangani (Ebuy et al. 2011). The third reserve is located near Masako (MA), a village 15 km north-east of

Kisangani (Mukinzi et al. 2005). All three reserves have a mixture of primary forest, secondary forest, fallow land and cropland. The soil in the three reserves is relatively similar and has clay content of 30 to 40%. The soil of the plateau of Yangambi is well described (Gilson et al. 1956).

Leaf traits

The first trait studied was the specific leaf area (SLA) or the ratio of leaf area to dry leaf mass which reflected leaf thickness, life span and the carbon economy of the tree (Wright et al. 2004). A second and related leaf trait studied was the leaf carbon content, which characterised the carbon economy of the leaf. Leaf nitrogen, phosphorus and $\delta^{15}N$ were included to reflect nutrient status of trees. The $\delta^{13}C$ was measured as a proxy of the water use efficiency. Nutrient concentrations were both expressed per leaf area and per dry leaf mass. Area-based traits reflected investments related to the leaf surface which represented the ability for light capture and were related to the trade-off between carbon gain and water loss through transpiration. Mass-based traits are so-called leaf economics that quantify revenues per unit of investment (Wright et al. 2004).

Research plots

Leaf materials were collected from seven circular plots with a radius of 7 m. The plots were situated in secondary tropical lowland forest and spread over three forest reserves (Table 1). Secondary forest was recognised based on the dominance of characteristic species such as *Macaranga* sp. or *Tetrorchidium didymostemon* (Lebrun & Gilbert 1954). In one of the plots (YO3), a typical primary rainforest species (*Gilbertiodendron dewevrei*) was dominant, although typical secondary forest species such as *Pycnanthus angolensis* were also present. Therefore, the YO3 plot should be considered as a more mature stage of forest succession. Plots were randomly selected at a minimal distance of 2 km from the nearest village. An inventory of all trees (excluding lianas) was made. All trees within the plot were felled. In total, 295 trees of 94 different species were identified. Wood density of several stem discs per tree was determined using the water displacement method, once in the field and once in the laboratory (Maniatis et al. 2011).

Table 1 Site characteristics of circular secondary forest plots (radius of 7 m) that were selected at the reserves of Yoko (YO), Yangambi (YA) and Masako (MA)

Parameter	Plot						
	YO1	YO2	YO3	YO4	YA	MA1	MA2
Latitude (°N)	00° 17'	00° 17'	00° 18'	00° 17'	00° 47'	00° 36'	00° 36'
Longitude (°E)	25° 17'	25° 17'	25° 16'	25° 17'	24° 29'	25° 13'	25° 13'
Elevation (m asl)	446	424	410	423	426	410	413
No. of species	18	22	18	23	12	18	27
Max tree height (m)	22.2	19.3	15.4	18.8	22.2	20.9	9.0
Basal area (m^2)	0.429	0.379	0.053	0.263	0.528	0.390	0.367
No. of trees	33 (6)	51 (6)	26 (7)	48 (19)	31 (11)	53 (27)	53 (12)

Number in parenthesis means the number of trees for which their leaves have been analysed; asl = above sea level; max = maximum

Leaf sampling

For each felled tree, a sample of 5 to 20 leaves (depending on leaf size), randomly distributed over the canopy, was collected. In this way, a range of possible different leaf ages was covered (clearly juvenile or senescent leaves were not included). All leaves of the subsamples collected at Yoko were delineated in the field on white paper. These drawings represented the original area of the leaves, before drying and shrinking. In Yangambi and Masako, a digital picture of the leaf was taken in the field on a white background with scale. Leaf area was determined by cutting and scanning the sketched leaves with a leaf area meter or by analysing the digital pictures as described by Rico-Garcia et al. (2009). Leaves were dipped in 96% alcohol to prevent rotting and moulding. To determine dry leaf weight and facilitate storage for further analysis, samples were partially dried (4 hours at 80 °C) at the University of Kisangani and fully dried (48 hours at 80 °C) upon return of the samples to the Laboratory of Plant Ecology at Ghent University.

Of the 295 initial leaf samples belonging to more than 90 species, mouldy samples were excluded. Subsequently, species without replicates were excluded from the overall analysis. The resulting dataset consisted of 88 samples from 10 species, belonging to five families (Table 2). All species have more than three replicates, except for *Ricinodendron heudelotii* with only two replicates. The species belonged to three shade tolerance classes: seven species were typical light demanding pioneering species, two species were shade tolerant and with *G. dewevrei* we included one ombrophile species typical of primary forest (Lebrun & Gilbert 1954).

Sample preparation and chemical analysis

The retained leaf samples were analysed in Ghent University. All samples for chemical analysis were finely ground and subsamples were prepared by weighing a given mass of crushed leaves. For phosphorus determination, subsamples of 0.5–1 g leaf material was prepared using the Chapman and Pratt (1961) procedure and were analysed for phosphorus P$_{mass}$ (mg P g^{-1} dry mass of leaf) using the autoanalyser method.

Analyses of the total foliar nitrogen and carbon contents and their stable isotope compositions were carried out using an elemental analyser coupled to an isotope ratio mass spectrometer. To ensure accuracy, duplicate subsamples of finely-ground leaf material in the range of 0.66–3.28 mg were weighed from each sample. The contents of leaf nitrogen and carbon were obtained on a percentage basis, which were converted to units of dry leaf mass—N$_{mass}$ and C$_{mass}$ (mg g^{-1}). Leaf ^{13}C/^{12}C and ^{15}N/^{14}N ratios were determined by EA-IRMS. Isotope ratios were expressed in delta notation relative to Vienna Pee Dee Belemnite standard for δ^{13}C and atmospheric air for δ^{15}N. A flour laboratory working standard was used with a known isotopic composition of -27.01‰ δ^{13}C and 2.69 ‰ δ^{15}N.

Statistical analysis

A principal component analysis (PCA) was performed using an orthogonal transformation to convert the set of possibly correlated leaf traits into a set of values of linearly uncorrelated variables called principle components. The PCA was conducted without coordinate rotation and only axes with an eigenvalue higher than one

Table 2　Overview of sampled species

Species	Code	Family	No.	Shade tolerance	Forest type	Phenology	D (cm)	H (m)	ρ (g cm⁻³)
Dichostemma glaucescens	Dg	Euphorbiaceae	13	T	Se/Pr	E	4.8 ± 1.3	6.4 ± 1.9	0.710 ± 0.052
Funtumia elastica	Fe	Apocynaceae	5	T	Se/Pr	E	4.6 ± 0.8	5.4 ± 2.2	0.702 ± 0.126
Gilbertiodendron dewevrei	Gd	Fabaceae	8	S	Pr	E	8.6 ± 10.7	8.7 ± 6.9	0.779 ± 0.030
Macaranga monandra	Mm	Euphorbiaceae	13	L	Se/Pr(gaps)	E	12.3 ± 11.7	11.6 ± 5.0	0.456 ± 0.052
Macaranga spinosa	Ms	Euphorbiaceae	15	L	Se/Pr(edges)	E	9.6 ± 5.2	11.0 ± 4.3	0.497 ± 0.076
Musanga cecropioïdes	Mc	Urticaceae	5	L	Se	E	19.7 ± 6.8	16.1 ± 2.9	0.470 ± 0.046
Oncoba welwitschii	Ow	Flacourtiaceae	5	L	Se	E/D	3.8 ± 1.2	6.0 ± 2.1	0.670 ± 0.001
Rauvolfia vomitoria	Rv	Apocynaceae	4	L	Se	E	3.9 ± 0.7	4.5 ± 1.9	0.641 ± 0.003
Ricinodendron heudelotii	Rh	Euphorbiaceae	2	L	Se/Pr(gaps)	D	20.5 ± 20	15.6 ± 9.3	0.392 ± 0.002
Tetrorchidium didymostemon	Td	Euphorbiaceae	18	L	Se/Pr(edges)	E	8.0 ± 2.9	8.3 ± 2.9	0.449 ± 0.024

L = light demanding; T = shade tolerant, S = ombrophile; D = diameter; H = height , ρ = wood density; Pr = primary, Se = secondary; E = evergreen, D = deciduous; source: Lebrun and Gilbert 1945, Schmelzer 2008

were retained. Bivariate relationships among and between traits and ordination scores were assessed with Spearman's correlation coefficient (r). Hierarchical clustering of the three PCA axes was performed using the Ward method to explore if distinct functional groups could be determined based on the observed leaf traits. One data point (for *T. didymostemon* at Yangambi) was not included in the PCA because it was a clear outlier for $\delta^{15}N$.

For comparisons of mean leaf traits, analysis of covariance (ANCOVA) was used. The data of the species *R. heudelotii* (n = 2) were removed for this analysis. First, we tested if the factor plot location had an effect for species with more than four replicates in each of the different forest reserves (*Macaranga monandra*: YA (n = 4) versus YO (n = 9); *Macaranga spinosa*: MA (n = 9) versus YA (n=4); *T. didymostemon*: MA (n = 11) versus YO (n = 6)). A multivariate ANCOVA was applied on the selected plots for these species separately. For variables that were not significantly influenced by location, all locations were pooled. Shade tolerance and species effects were analysed with ANCOVA with the species (n = 9) factor nested in the shade tolerance factor (n = 3) and with tree height as co-variable. Homoscedasticity was checked with the Levene test. Trait variables that violated the homoscedasticity assumption were root transformed (N_{mass}, P_{mass}) or ln transformed (N_{area}, P_{area}, C_{area}).

RESULTS

Principal component analysis

The leaf thickness, nitrogen and phosphorus status as described by the major components of the first three PCA axes that were retained explained 42.9, 27.6 and 11.8% of the total variance within the dataset (results not shown). Specifically, the PCA1 axis showed highly significant (p < 0.01) negative correlation with SLA and positive correlations with area-based concentrations of nitrogen, phosphorus and carbon (Table 3). The first axis (PCA1) could be interpreted as the axis representing leaf thickness where higher positive values represented thicker leaves. The second axis (PCA2) showed strong negative correlation with the C:N ratio (p < 0.01) and strong positive correlation with N_{mass} and $\delta^{15}N$ (p < 0.01). Additionally, a significant

Table 3   Spearman's correlation coefficients between ordination scores and leaf traits

Parameter	PCA-axis		
	1	2	3
$\delta^{15}N$	0.17	**0.74**	**-0.30**
N_{mass}	-0.21	**0.90**	0.12
$\delta^{13}C$	**0.62**	**0.34**	0.00
C_{mass}	**0.64**	-0.26	-0.24
P_{mass}	0.01	**0.59**	**0.67**
N:P	-0.12	0.13	**-0.92**
C:N	0.32	**-0.89**	-0.08
SLA	**-0.81**	0.49	-0.14
C_{area}	**0.87**	-0.45	-0.13
N_{area}	**0.95**	-0.07	-0.07
P_{area}	**0.88**	-0.08	**0.33**
H	**0.53**	0.07	-0.02
D	**0.49**	0.20	0.01
ρ	-0.09	**-0.39**	-0.11

Leaf ^{15}N composition ($\delta^{15}N$), mass-based nitrogen concentration (N_{mass}), ^{13}C composition ($\delta^{13}C$), mass-based carbon concentration (C_{mass}), mass-based phosphorus concentration (P_{mass}), specific leaf area (SLA), area-based nitrogen concentration (N_{area}), area-based carbon concentration (C_{area}) area-based phosphorus concentration (P_{area}), tree height (H), diameter (D) and wood density (ρ); statistical significant scores (p < 0.01) are shown in bold

but less strong correlation with P_{mass} should be mentioned. This axis could be interpreted as a representation of the N status, with high values indicating higher N content. The third axis (PCA3) showed positive correlation with P_{mass} and highly negative correlation with the N:P ratio. This axis represented the P status, with high values for leaves with a high P content.

Musanga cecropioïdes had thicker leaves compared with other species (Figure 1). There were clear species-based differences in leaf N content. In comparison with *G. dewevrei*, which had low leaf N content, the high PCA2 values for *T. didymostemon* and *Oncoba welwitschii* indicated high leaf N content. The data also indicated significantly lower P concentrations compared with other species for *Dichostemma glaucescens* given the lower PCA3 values. The ombrophile species (i.e. *G. dewevrei*) showed relatively thicker leaves with low N content.

Four cluster groups were distinguished by the hierarchical clustering of the PCA scores (Figure
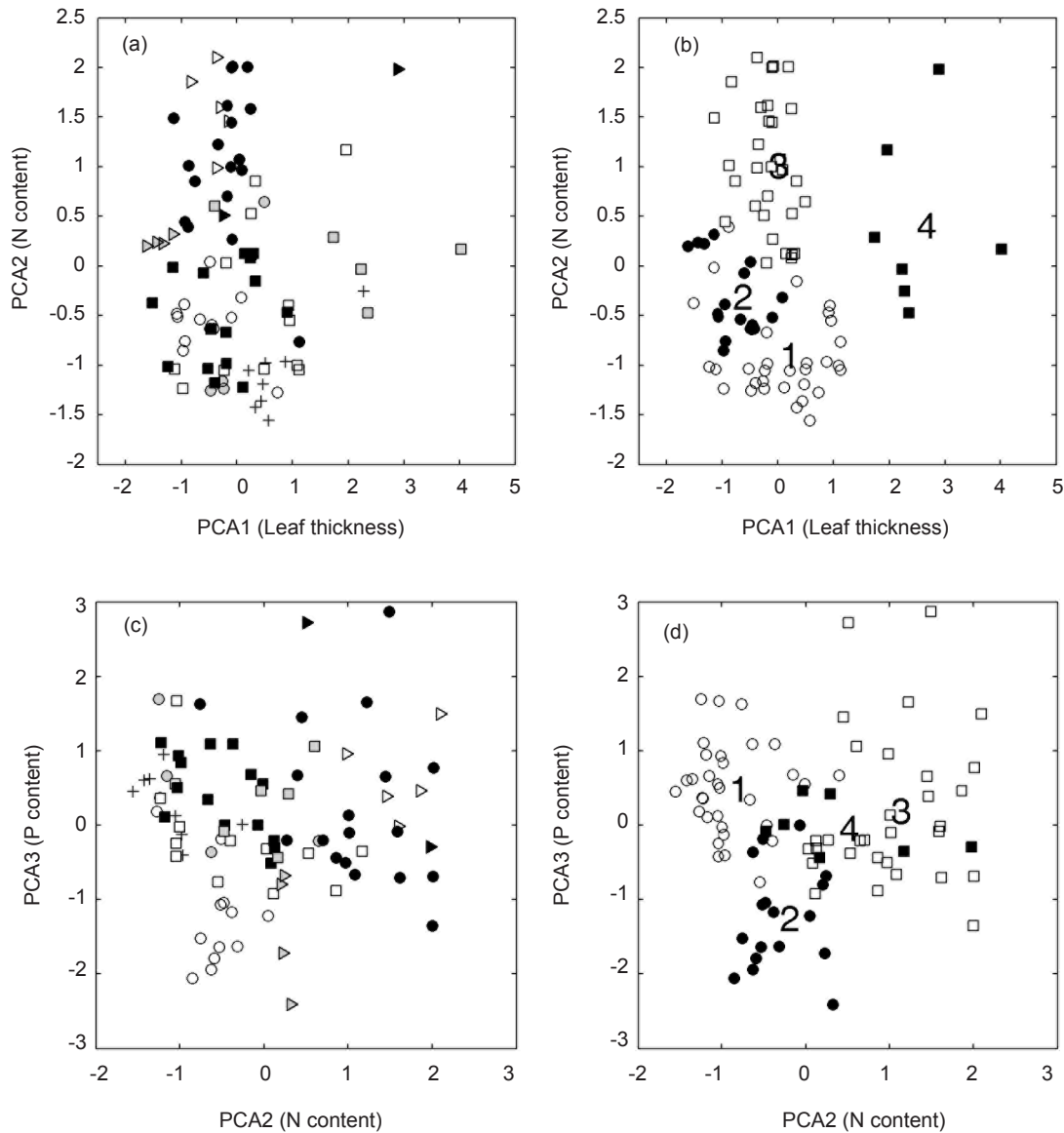
Figure 1   Principle component ordination considering 11 leaf traits along (a and b) PCA axes 1–2 and (c and d) PCA axes 2 and 3; symbols show species (a, c) or cluster groups (b, d); species: *Dichostemma glaucescens* (open circles), *Funtumia elastica* (grey circles), *Gilbertiodendron dewevrei* (crosses), *Musanga cecropioïdes* (grey squares), *Macaranga monandra* (open squares), *Macaranga spinosa* (black squares), *Oncoba welwitschii* (open triangles), *Rauvolfia vomitoria* (grey triangles), *Ricinodendron heudelotii* (black triangles), *Tetrorchidium didymostemon* (black circles); numbers show average ordination score for each cluster group: 1 (open circles), 2 (closed circles), 3 (open squares) and 4 (closed squares)

1b and d). Group 1 represented samples of leaves with average thickness, low N concentration and variable P concentration. Group 2 contained leaf samples with relatively thin leaves, average N content but low P content. Leaves with average thickness and high N and P contents belonged to group 3. Group 4 represented samples with thick leaves with variable N content and average P content.

Correlations among leaf traits

High correlations were found between variables that were derived from each other (e.g. between N_{mass} and C:N ratio or between SLA and area-based concentrations; Table 4). Among variables that were measured independently from one another, several significant correlations were found. A significant correlation existed between

Table 4 Matrix showing correlations (Spearman's coefficient) among the leaf traits, tree height (H), diameter (D) and wood density (ρ)

	H	D	ρ	$\delta^{15}N$	N_{mass}	$\delta^{13}C$	C_{mass}	P_{mass}	N:P	C:N	SLA	C_{area}	N_{area}	P_{area}
H	1	0.89**	-0.42**	0.08	-0.07	0.69**	0.29**	0.04	-0.08	0.13	-0.36**	0.44**	0.44**	0.41**
D		1	-0.51**	0.14	0.06	0.74**	0.20	0.15	-0.10	0.01	-0.25*	0.34**	0.40**	0.38**
ρ			1	-0.10	-0.39**	-0.50**	0.23*	-0.38**	0.11	0.39**	-0.14	0.13	-0.06	-0.13
$\delta^{15}N$				1	0.51**	0.24*	-0.02	0.33**	0.16	-0.49**	0.20	-0.16	0.09	-0.02
N_{mass}					1	0.18	-0.26*	0.74**	-0.02	-0.98**	0.60**	-0.56**	-0.19	-0.12
$\delta^{13}C$						1	0.26*	0.23*	-0.11	-0.09	-0.30**	0.35**	0.49**	0.49**
C_{mass}							1	0.02	-0.26*	0.42**	-0.51**	0.61**	0.51**	0.54**
P_{mass}								1	-0.62**	-0.69**	0.38**	-0.36**	-0.03	0.25**
N:P									1	-0.02	-0.01	0.10	-0.03	-0.44**
C:N										1	-0.66**	0.65**	0.28**	0.21*
SLA											1	-0.94**	-0.82**	-0.70**
C_{area}												1	0.88**	0.75**
N_{area}													1	0.89**
P_{area}														1

Leaf ^{15}N composition ($\delta^{15}N$), mass-based nitrogen concentration (N_{mass}), ^{13}C composition ($\delta^{13}C$), mass-based carbon concentration (C_{mass}), mass-based phosphorus concentration (P_{mass}), specific leaf area (SLA), area-based carbon concentration (C_{area}), area-based nitrogen concentration (N_{area}), area-based phosphorus concentration (P_{area}); * p < 0.05, ** p < 0.01

N_{mass} and P_{mass} (r = 0.74, p < 0.01). To a lesser degree, SLA and N_{mass} were correlated (r = 0.60, p < 0.01). Weaker but significant correlations were found between $\delta^{13}C$ and N_{area} (r = 0.49, p < 0.01) and between $\delta^{15}N$ and C:N (r = -0.49, p < 0.01).

Location and species effect

The multivariate ANCOVA that was applied separately on three selected species with enough replicates revealed that the only variable that was significantly influenced by location was $\delta^{15}N$ (result not shown). Average values for $\delta^{15}N$ for the selected species were higher in Yangambi (5.85 ± 0.17 for Mm, 4.52 ± 0.36 for Ms) than in Yoko (2.44 ± 2.02 for Mm) and Masako (1.78 ± 0.91 for Ms) (results not shown). Therefore $\delta^{15}N$ was not used for studying the species and height effects. Shade tolerance was a significant factor for all other leaf traits but species was a significant factor within each shade tolerance class. Mean values for each trait per species are given in Table 5. *Tetrorchidium didymostemon* and *O. welwitschii* had significantly higher values of N_{mass} and P_{mass} than the other species. For N:P ratio, *D. glaucescens* and *Rauvolfia vomitoria* had significantly higher values. Groupings for other variables were more complex.

Relations with tree height, diameter and wood density

Tree height had significant influence on $\delta^{13}C$ (r = 0.69), SLA (r = -0.36) and concentrations of N (r = 0.44), P (r = 0.41) and C (r = 0.44) expressed per area (p < 0.01) (Table 4). The PCA1 axis was significantly positively correlated with tree height and diameter (Table 3). Taller trees had less negative $\delta^{13}C$ values and lower SLA values (Figure 2). Wood density was negatively correlated with PCA2 (Table 3), the N content axis. This negative relation between wood density and leaf nutrient content was also obvious from the negative correlations with N_{mass} (r = -0.39, p < 0.01) and P_{mass} (r = -0.38, p < 0.01) (Table 4). Nevertheless, the leaf trait that showed the strongest correlation with wood density was leaf $\delta^{13}C$ content (r = -0.50, p < 0.01) (Table 4).

DISCUSSION

The observed average leaf trait values of this study were consistent with global datasets (Kattge et al. 2011) and literature values for tropical forest (Fyllas et al. 2009). Values of leaf nitrogen and phosphorus per dry mass were similar to the values found for a secondary forest in Cameroon (Meir et al. 2001). However, these values were at the higher side of the global range (Kattge et al. 2011) and values for the Amazon (Fyllas et al. 2009). The N:P ratio in this study (18.4 ± 3.4) was clearly higher than the global average (12.8) (Kattge et al. 2011), indicating a high abundance of nitrogen and limiting availability of phosphorus. The N:P ratio was larger than 16 for all species, indicating that all of them were P limited. These forests clearly produce high quality litter (with low C:N), which is important for nutrient recycling.

Most observed relations between individual traits were consistent with previous studies in tropical regions. The correlation between N_{mass} and P_{mass} confirms measurements in tropical forests over all continents (Kenzo et al. 2004, Domingues et al. 2010, Patiño et al. 2012). The consistent correlation between leaf N and P showed that the ratio between nitrogen and phosphorus limitation was similar for all sampled trees and locations. The positive relation between SLA and leaf N is a trend observed for many species in several biomes (Reich et al. 1998). In addition, our results illustrated the different functionality of area-based versus mass-based traits. Mass-based traits were mainly correlated with one another, with wood density and with $\delta^{15}N$. Area-based traits reflect photosynthetic capacity and the adaptation to light availability and are therefore more correlated with height, diameter and $\delta^{13}C$.

The only leaf trait that was significantly influenced by plot location was $\delta^{15}N$. Leaf $\delta^{15}N$ is suggested to be an indicator of ecosystem N cycling, with higher values indicating greater N availability and a more open N cycle (Pardo et al. 2006). However, leaf $\delta^{15}N$ is the result of a complex interplay between different factors such as soil N availability, internal N cycling, species composition (N fixating species), landuse history, climate and mycorrhizal symbioses (Pardo et al. 2006). This makes the interpretation of the natural $\delta^{15}N$ abundance in plants not straightforward. Additional data on soil composition, biological N fixation and landuse history are needed to explain the observed patterns in leaf $\delta^{15}N$. Nevertheless, the leaf $\delta^{15}N$ values for all species were lower than the $\delta^{15}N$ values (7.01 to 9.62%; results not shown) observed in the top

Table 5 Mean leaf trait values (± standard deviation) for all species

Parameter	Dg	Fe	Gd	Mc	Mm	Ms	Ow	Rv	Td	Average
N_{mass} (mg g⁻¹)	21.5 ± 0.8 a	23.8 ± 1.3 a	20.6 ± 0.9 a	25.0 ± 1.4 a	22.6 ± 0.8 a	23.4 ± 0.7 a	35.9 ± 1.6 b	25.4 ± 1.5 a	31.4 ± 0.8 b	25.5 ± 5.6
$\delta^{13}C$ (%)	-33.9 ± 0.3 a	-33.4 ± 0.4 ab	-34.3 ± 0.3 a	-31.4 ± 0.5 b	-32.4 ± 0.3 b	-32.9 ± 0.3 ab	-33.3 ± 0.4 ab	-32.6 ± 0.5 ab	-32.1 ± 0.2 b	-32.9 ± 1.7
C_{mass} (mg g⁻¹)	441 ± 4 bc	459 ± 6 c	458 ± 4 c	461 ± 6 c	443 ± 3 c	445 ± 3 c	455 ± 6 c	411 ± 6 a	427 ± 3 ab	445 ± 18
P_{mass} (mg g⁻¹)	0.94 ± 0.06 a	1.35 ± 0.11 a	1.27 ± 0.11 a	1.58 ± 0.12 a	1.31 ± 0.07 a	1.43 ± 0.06 a	2.14 ± 0.13 b	1.03 ± 0.10 a	1.86 ± 0.06 b	1.44 ± 0.45
N:P	22.9 ± 0.7 b	17.7 ± 1.1 a	16.3 ± 0.8 a	16.0 ± 1.2 a	17.4 ± 0.7 a	16.5 ± 0.6 a	16.8 ± 1.1 a	24.6 ± 1.2 b	17.2 ± 0.6 a	18.4 ± 3.4
C:N	20.5 ± 0.6 cd	19.4 ± 0.9 bcd	22.2 ± 0.7 d	18.6 ± 1.0 bc	19.8 ± 0.6 bcd	19.1 ± 0.5 bc	12.9 ± 0.9 a	16.2 ± 1.0 ab	13.9 ± 0.5 a	18.1 ± 3.6
SLA (10^{-3} m² g⁻¹)	19 ± 2 ab	18 ± 3 ab	10 ± 2 a	20 ± 3 abc	18 ± 2 ab	24 ± 2 bc	32 ± 3 c	32 ± 3 c	25 ± 2 bc	22 ± 9
C_{area} (g m⁻²)	24.2 ± 2.1 bcd	29.2 ± 4.0 bcde	42.3 ± 4.5 e	34.8 ± 5.1 de	27.1 ± 2.3 cde	19.4 ± 1.5 abc	16.3 ± 2.2 abc	13.6 ± 2.1 a	18.0 ± 1.3 ab	25.0 ± 14.2
N_{area} (g m⁻²)	1.18 ± 0.09 abc	1.51 ± 0.18 bcd	1.90 ± 0.17 d	1.88 ± 0.24 cd	1.38 ± 0.10 abcd	1.02 ± 0.07 ab	1.28 ± 0.15 abcd	0.84 ± 0.11 a	1.31 ± 0.08 abc	1.37 ± 0.65
P_{area} (mg m⁻²)	51.8 ± 4.1 ab	85.9 ± 10.4 cde	117.3 ± 11.0 de	118.0 ± 15.4 e	79.5 ± 6.0 cde	62.3 ± 4.3 bc	76.6 ± 9.2 bcde	34.1 ± 4.7 a	77.4 ± 4.8 ce	78.1 ± 39.3
$\delta^{15}N$ (%)	4.46 ± 3.77	2.06 ± 1.98	2.97 ± 1.29	3.25 ± 1.38	3.49 ± 2.32	2.69 ± 1.46	6.61 ± 1.18	2.71 ± 0.79	4.80 ± 1.78	3.76 ± 2.34

Dg (*Dichostemma glaucescens*), Fe (*Funtumia elastica*), Gd (*Gilbertiodendron dewevrei*), Mc (*Musanga cecropioïdes*), Mn (*Macaranga monandra*), Ms (*Macaranga spinosa*), Ow (*Oncoba welwitschii*), Rv (*Rauvolfia vomitoria*), Rh (*Ricinodendron heudelotii*), Td (*Tetrorchidium didymostemon*); differences in means were established with ANCOVA, significance level p < 0.05, values followed by the same letters in the same row are not significantly different
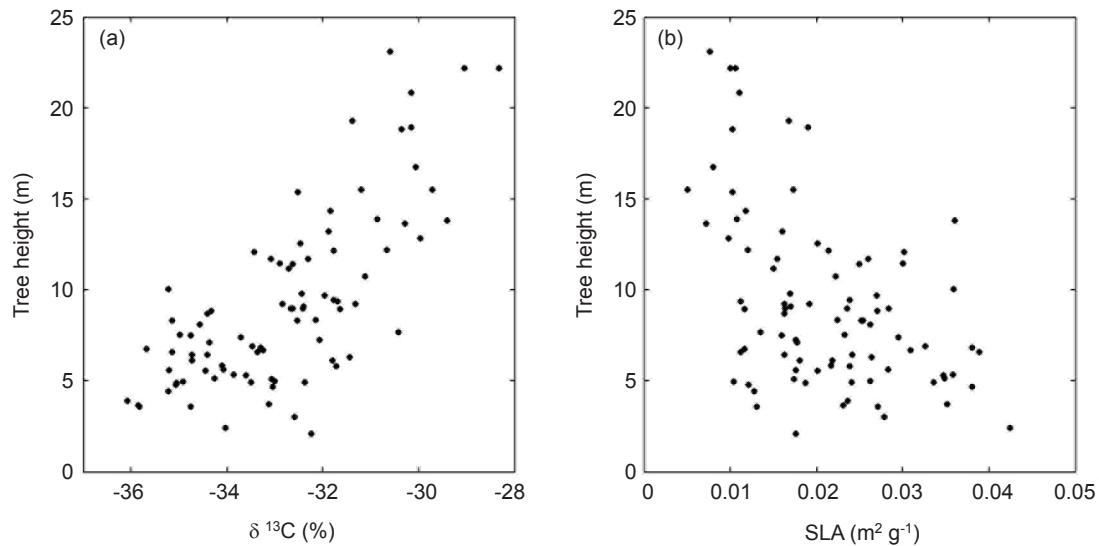
Figure 2   Scatter plots representing the vertical profiles of leaf traits (a) δ^{13}C and (b) specific leaf area (SLA) along the tree height gradient between individual trees

soil (0–10 cm) in all plots. This indicates that at least a fraction of the N uptake of trees is from biological N fixation from the air or provided by mycorrhiza.

The observed vertical canopy gradients in this study were between-tree gradients based on differences in tree height between individual sampled trees. Our results indicated that higher trees had thicker sun-adapted leaves. The correlations observed between height and several leaf traits (δ^{13}C, C_{mass}, N_{area} and P_{area}) are confirmed by data from the Amazon (Lloyd et al. 2010).

The species factor was significant in our analysis. Moreover, species within the same shade tolerance class (e.g. *M. cecropioïdes* and *M. spinosa*) fell into different cluster groups. This illustrates that shade tolerance is not the only factor determining the functional diversity. We found high leaf trait diversity among the secondary forest species leading to four species groups that could be considered as four different functional niches. The PCA axes reflect the classical leaf economic spectrum (Wright et al. 2004), ranging from species with a slow return on investment (thicker leaves with low nutrients content and a long life span) to species with a quick return on investment (thinner leaves with high nutrient concentrations and a short lifespan). Light-demanding species had significantly higher N content (PCA2), indicating the typical pioneering character of these species. The ombrophile species had significantly thicker leaves (PCA1), typical for shade-adapted leaves with higher leaf

longevity. The significant negative correlation between wood density and N content (PCA2) confirms previous findings that species with slow return on investment are typically the species with low growth rates and high wood density, which represent a more conservative strategy of resource use and turnover (Reich et al. 2003).

The observed functional diversity among the different sampled species showed that within the secondary tropical forests in the Congo Basin, multiple species coexisted with diverse strategies occupying different niches. In addition, certain plant traits are co-varying non-randomly, reflecting general ecological strategies valid for all functional groups within these ecosystems.

These diverse resource-use strategies of individual species should be investigated in more detail in future. Additional key traits such as rooting depth, nitrogen fixing abilities, interactions with mycorrhiza, light use strategy, dark respiration, photosynthetic capacity and leaf life span should be measured, if logistics allow doing so. Measuring within-tree gradients will be an important next step to see if the observed between-tree vertical gradients are confirmed.

It is clear that many more leaf trait data are needed for the Congo Basin. Future research should expand the sampling to a wider range of sites covering multiple forest and soil types during different periods within multiple years. The characterisation of plant functional traits within the Congo Basin forest is vital for our understanding of key ecological processes.

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REFERENCES

Chapman HD & Pratt FP. 1961. Ammoniumvandate-molybdate method for determination of phosphorus. Pp 184–203 in *Methods of Analysis for Soils, Plants and Water*. University of California, Riverside.

De Wasseige C, Devers D, de Marcken P, Eba'a Atyi R, Nasi R & Mayaux PH. 2010. *The Forests of the Congo Basin—State of the Forest 2008.* Publications Office of the European Union, Luxembourg.

Domingues TF et al. 2010. Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. *Plant, Cell and Environment* 33: 959–980.

Ebuy J, Lokombe JP, Ponette Q, Sonwa D & Picard N. 2011. Allometric equation for predicting aboveground biomass of three tree species. *Journal of Tropical Forest Science* 23: 125–132.

FAO. 2011. The state of forests in the Amazon Basin, Congo Basin and Southeast Asia. A report prepared for the Summit of the Three Rainforest Basins Brazzaville, Republic of Congo. Food and Agriculture Organization, Rome.

Fyllas NM et al. 2009. Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate. *Biogeosciences* 6: 2677–2708.

Gilson P, Van Wambeke A & Gutzwiller R. 1956. *Carte des Sols et de la Végétation du Congo Belge et du Ruanda-Urundi. Yangambi (Planchette 2: Yangambi)*. Institut National Pour L'étude Agronomique du Congo Belge, Yangambi.

Kattge J et al. 2011. TRY—a global database of plant traits. *Global Change Biology* 17: 2905–2935.

Kenzo T, Ichie T, Yoneda R, Kitahashi Y, Watanabe Y, Ninomiya I & Koike T. 2004. Interspecific variation of photosynthesis and leaf characteristics in canopy trees of five species of Dipterocarpaceae in a tropical rain forest. *Tree Physiology* 24: 1187–1192.

Lebrun J & Gilbert G. 1954. *Une Classification Écologique des Forêts du Congo*. Série Scientfique 63. INEAC, Yangambi.

Lloyd J et al. 2010. Optimisation of photosynthetic carbon gain and within-canopy gradients of associated foliar traits for Amazon forest trees. *Biogeosciences* 7: 1833–1859.

Maniatis D, Saint André L, Temmerman M, Malhi Y & Beeckman H. 2011. The potential of using xylarium wood samples for wood density calculations: a comparison of approaches for volume measurement. *iForest* 4: 150–159.

Meir P, Grace J & Miranda AC. 2001. Leaf respiration in two tropical rainforests: constraints on physiology by phosphorus, nitrogen and temperature. *Functional Ecology* 15: 378–387.

Mukinzi I, Katuala PGB, Kennis J, Gambalemoke M, Kadange N, Dudu AM, Colyn M & Huterer R. 2005. Preliminary data on the biodiversity of rodents and insectivores (mammalia) in the periphery of Kisangani (DR Congo). *Belgian Journal of Zoology* 135: 133–140.

Pardo LH et al. 2006. Regional assessment of N saturation using foliar and root delta(15)N. *Biogeochemistry* 80: 143–171.

Patiño S, Fyllas NM, Baker TR, Paiva R, Quesada CA, Santos AJB, Schwarz M, ter Steege H, Phillips OL & Lloyd J. 2012. Coordination of physiological and structural traits in Amazon forest trees. *Biogeosciences* 9: 775–801.

Peel MC, Finlayson BL & McMahon TA. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences* 11: 1633–1644.

Reich PB, Ellsworth DS & Walters MB. 1998. Leaf structure (specific leaf area) modulates photosynthesis–nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology* 12: 948–958.

Reich PB, Wright IJ, Cavender-Bares J, Craine M, Oleksyn J, Westoby M & Walters MB. 2003. The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* 164: 143–164.

Rico-Garcia E, Hernandez-Hernandez F, Soto-Zarazua GM & Herrera-Ruiz G. 2009. Two new methods for the estimation of leaf area using digital photography. *International Journal of Agriculture and Biology* 11: 397–400.

Schmelzer GH. 2008. Record from Protabase. Http://database.prota.org/search.htm.

Sterck F, Markesteijn L, Schieving F & Poorter L. 2011. Functional traits determine trade-offs and niches in a tropical forest community. *Proceedings of the National Academy of Sciences*. Doi 10.1073/pnas.1106950108.

Verbeeck H, Boeckx P & Steppe K. 2011. Tropical forests: include Congo Basin. *Nature* 479: 179.

Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I & Garnier E. 2007. Let the concept of trait be functional! *Oikos* 116: 882–892.

Wright IJ et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.