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Dear *Biogeosciences* editor,

We hereby submit our revised manuscript entitled "Parallel functional and stoichiometric trait shifts in South-American and African forest communities with elevation" to be considered for publication as a research article in *Biogeosciences*.

We would like to thank both reviewers and the editor for the thoughtful comments, fast handling, and positive and constructive assessments of our manuscript. We believe we have addressed and clarified each comment made by both reviewers. The detailed responses are below, and the line numbers refer to the revised manuscript with track changes, which is also below.

We feel that the revised manuscript has improved again and hope that its changes satisfy both reviewers and the editor.

Yours sincerely,

The authors

## Responses to Reviewers

Dear Dr. Bauters and co-authors,  
the two reviewers are satisfied with your revisions and have some suggestions for further improvement.

*We would like to thank the handling editor for the efforts made on this manuscript. The review process has been fast and constructive, and we feel that we were able to improve the manuscript substantially from the earlier version.*

In addition to their comments I ask you to take care of the following:

\* Key words: I agree that „elevational gradient“ is more appropriate than „altitudinal gradient“, and suggest to delete „elevational transects“, which overlaps significantly

*We have now adapted this in the new MS version (p1 L16).*

\* l. 75 To my understanding elevational gradients typically do imply changes in growing season length. Please correct / delete this part of the statement.

*We have now adapted this in the new MS version (p3 L48).*

\* l. 100 add reference

*We have now adapted this in the new MS version (p4 L74-75).*

\* l. 242 your reasoning is hard to follow: why should it be nonsensical to report on and test for these abiotic drivers in your study?

*This was indeed poorly formulated. We have now adapted this in the MS. It would be sensical – interesting even- to test for abiotic drivers, but in our opinion it would be an overkill of the dataset that we report on. A more elaborate analysis with direct abiotic changes instead of altitude as explanatories would be interesting in a larger meta-analysis with a number of transects. Additionally, we don't have field estimates of these drivers in the sites (p10 L214-217).*

\* Table 2: add unit for SLA

*We have now adapted this in the new MS version (p37 Table2).*

Please address these remaining issues and carefully correct the typos in a revision, and explain in your response letter how you have dealt with the comments and what changes you have made to the manuscript.

Best regards,  
Michael Bahn

## Reviewer report 1

In my opinion the authors have substantially improved the manuscript and addressed most of the concerns I had in the original version.

*We would like to thank the reviewer once more for all the efforts on this manuscript and apologize for the unclear points that remained after the previous review round. We feel that his/her time and efforts have substantially improved this MS.*

There are some issues with their response, however. Namely, the authors do not provide accurate line numbers for the new MS so that I can go check what they claim to have addressed. Do they give the old line numbers again or did they not go back and correct the line numbers during revision?

*The line numbers that we provided corroborated with the changes of the version with track changes that was provided with the response letter. We apologize for this unclarity and*

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*acknowledge that we should have stated this explicitly in the response letter. The line numbers in the current revisions again refer to the revised MS with track changes below.*

Response to L203: What do you mean strong focus on Africa? Half of your data is from Ecuador and the datasets you compare to are from all over. My original comment was about your description of the methods used to select the single mountain transects that you compare your data to, which I think is poorly justified/explained in the MS. Saying it would be too much work to bring in more data is not a valid excuse. You once again cite an argument in Tanner 1998 that doesn't really support the decision you've made here - to use only single mountain transects because you can find high or low nutrient soils on any mountain? So why not use data from multiple mountains that would alleviate the chance that one was from an unusually low or high nutrient soil?

*We appreciate the reviewer's vision on this, and acknowledge that our formulated response may have been poorly expressed. We have now deleted Tanner's argument from the MS (p11 L234-237). But in the end, this might also be a point we will have to respectfully agree to disagree upon. As we explain in the MS, we add these studies where community-weighted averages of traits are reported from similar setups to compare to our study. We therefore only leave out the study by Asner et al., because he assembled community-weighted averages from plots all over the tropics and then looked at elevation as a driving factor for the changes noted in his dataset. As we acknowledge in the MS, this is an interesting study, but it uses a different approach as the one we, and others, use. If we were to include this study, where would we draw the line? Then we might include all the published data on community weighted average of any plot in the tropics. We prefer not to do this, not because of the workload, but because:*

- 1) The goal of this study is to report new findings on two different elevation transects. To frame these findings, we compare them to existing reports with a similar setup. Doing a bigger meta-analysis is a different study, and would get the focus off our results.*
- 2) As we stated in the previous response, there are other factors that might be confounding in such meta-analyses (atmospheric N deposition seasonality, etc.). Additionally, data of our two transects show the same slope but a different intercept. If we would pick plots randomly from both transects to add in such a meta-analysis; it would not be likely to find the same slopes (or maybe not even trends) while when looking at 'same mountain' trends the opposite is true.*

You again invoke the type or degree of mycorrhization to possibly explain your 15N trends without actually discussing if any small proportion of your tree species associate with EcM.

*We simply don't have data on EcM association, so we cannot confirm that this is/is not causing divergence in soil-canopy  $\delta^{15}N$ . However, the shift in enrichment factor, if real, is likely caused by either a shift in plant N source, or a shift in mycorrhizal association. Hence we did not want to leave this "untouched" in the MS. We do acknowledge that we don't have EcM data in the MS.*

The manuscript will benefit greatly from careful editorial work as there are typically 3-4 typos per page.

*We apologize for this, and have now carefully re-edited the MS.*

## **Reviewer report 2**

The authors have adequately addressed my previous comments, and I only have few minor comments.

*We would like to thank the reviewer for both review efforts. We acknowledge that his time and efforts have substantially improved the manuscript.*

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The fact that the study is based on two transects only should be mentioned in abstract, so far it is unclear how many transects were used.

*We have now adapted this in the new MS version (p2 L26).*

Line 94 – “therefore” instead of “therefor”

*We have now adapted this in the new MS version (p4 L67).*

Line 280-281 – split into two sentences

*We have now adapted this in the new MS version (p12 L 248).*

Table 2 in Appendix should be perhaps Table S3?

*Thank you for this. We have now adapted this in the new MS version. (See supplementary information, and MS p15 L 323-324)*

The discussion is still a bit hard to follow for me and it would be helpful to stress the main findings and broader impact in the first paragraph.

*We have now included a short 'introductory' paragraph at the beginning of the discussion. (p9 L191-197)*

1 Parallel functional and stoichiometric trait shifts in South-American and African forest  
2 communities with elevation

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16 Keywords : tropical forests, leaf traits, ~~elevational transects~~, canopy chemistry, elevational  
17 gradient, cross-continental comparison, leaf economics spectrum

18 Short Title: Parallel trait shifts in South-American and African elevational forest transects

19 Word count Abstract: 177

20 Word count Body: 3164

21 Numbers of references: 47

22 Abstract

23 The Amazon and Congo basin are the two largest continuous blocks of tropical forest with a central  
24 role for global biogeochemical cycles and ecology. However, both biomes differ in structure and  
25 species richness and composition. Understanding future directions of the response of both biomes  
26 to environmental change is paramount. We used one elevational ~~gradients~~gradient on both  
27 continents to investigate functional and stoichiometric trait shifts of tropical forest in South  
28 America and Africa. We measured community-weighted functional canopy traits and canopy and  
29 topsoil  $\delta^{15}\text{N}$  signatures. We found that the functional forest composition response along both  
30 transects was parallel, with a shift towards more nitrogen conservative species at higher elevations.  
31 Moreover, canopy and topsoil  $\delta^{15}\text{N}$  signals decreased with increasing altitude, suggesting a more  
32 conservative N cycle at higher elevations. This cross-continental study provides empirical  
33 indications that both South-American and African tropical forest show a parallel response with  
34 altitude, driven by nitrogen availability along the elevational gradients, which in turn induces a  
35 shift in the functional forest composition. More standardized research, and more research on other  
36 elevational gradients is needed to confirm our observations.

37

38

39

## 40 Introduction

41 A good understanding of the future response of tropical forest ecosystems to global change is  
42 required because of their vital role in global biogeochemical cycles and ecology. However, due to  
43 the long turnover times in forest ecosystems, it is hard to acquire insight in these future responses.  
44 As a result, empirical research has since long turned to studying ecosystems along natural  
45 gradients, which can greatly advance our understanding of ecosystem ecology and function in  
46 response to environmental shifts. Elevational gradients in particular offer open-air space-for-time  
47 experiments. Contrary to latitudinal gradients or elevational gradients in the higher latitude zones,  
48 they are not complicated by changes in seasonality ~~or growing season length~~, and with careful  
49 interpretation can offer great insights in tropical forest functioning (Körner, 2007; Malhi et al.,  
50 2010; Sundqvist et al., 2013). Hence, elevational transects have been postulated as a viable and  
51 useful setup to assess long-term ecosystem responses to environmental changes, and serve as an  
52 empirical tool to assess future trajectories of forest ecosystems under global change (Malhi et al.,  
53 2010; Sundqvist et al., 2013). This has invoked research efforts on transects in South America, but  
54 no such studies have been carried out in central African forests, leaving the second-largest  
55 continuous block of tropical forest understudied. Nevertheless, recent work has shown that African  
56 and South-American tropical forest currently show important differences in structure (Banin et al.,  
57 2012) and species richness and composition (Slik et al., 2015). These differences call for cross-  
58 continental empirical research in both the Amazon and the Congo basin (Corlett and Primack,  
59 2006), and in this context we can raise questions about the universality of tropical forest  
60 biogeochemistry and functioning across both continents, and subsequently their response to future  
61 global change scenarios. Additionally, due to the central role of nutrient availability that drives  
62 both net ecosystem productivity (NEP) and ecosystem carbon use efficiency (CUEe ) (Fernandez-

63 Martinez et al., 2014), the effect of climatic gradients on nutrient availability should be better  
64 understood.

65

66 Indeed, recent efforts have shown that biosphere-atmosphere carbon exchange in forests is  
67 regulated by nutrient availability (Fernandez-Martinez et al., 2014) and ~~therefor~~therefore, changes  
68 in nutrient bio-availability induced by global change need to be accounted for. Canopy chemical  
69 traits are proxies that are relatively easy to assess, and from which ecosystem functioning and  
70 biogeochemistry can be inferred (Asner et al., ~~2015a~~2015; Wright et al., 2004). Nutrient ratios and  
71 concentrations in leaves, along with specific leaf area (SLA), are traits that are known to cluster  
72 around the leaf economic spectrum, which expresses a trade-off in photosynthetic efficiency and  
73 leaf turnover. Indeed, canopy ~~nitrogen (N) and phosphorus (P)~~nutrients play key roles in  
74 photosynthesis, and are hence vital for carbon exchange processes at the leaf level- (Evans, 1989;  
75 Reich et al., 2009). Consequently, species with high SLA, N and P are associated with high  
76 photosynthesis rates (Poorter et al., 2009; Reich et al., 1997; Wright et al., 2004), but have an  
77 ‘expensive’ nutrient economy (fast leaf turnover). Previous work has shown that these traits vary  
78 systematically with landscape biogeochemistry (Asner et al., 2014, ~~2015b~~2015) and hence the  
79 functional canopy signature of forests across gradients express the ecological response to changes  
80 in nutrient availability. Canopy chemistry has received increasingly more attention because of its  
81 inherent link to the plant strategy. Nevertheless, and as rightfully noted by Asner and Martin  
82 (2016), there are only limited surveys on canopy functional signatures in the tropics, while this  
83 information is vital for a landscape-scale understanding of tropical forest assembly. In addition to  
84 leaf traits, both leaf and soil  $\delta^{15}\text{N}$  are known integrators of the local N-cycle and analysis of natural  
85 abundance of stable N isotope ratios is a powerful and extensively studied proxy for N cycling in

86 ecosystems (Högberg, 1997). Previous efforts have shown that shifts towards lower  $\delta^{15}\text{N}$  values  
87 indicate a more closed N-cycle with lower N availability, and *vice versa* (Brookshire et al., 2012;  
88 Craine et al., 2015; Houlton et al., 2006). This shift in isotopic ratios is caused by increased rates  
89 in fractioning processes such as denitrification, where  $^{14}\text{N}$  is preferentially consumed, leaving the  
90 source pool enriched with  $^{15}\text{N}$  (Hobbie and Ouimette, 2009). Hence  $\delta^{15}\text{N}$  values have been used  
91 to infer shifts in N openness across natural gradients (Martinelli et al., 1999; Menge et al., 2011;  
92 Vitousek et al., 1989). Hence), and subsequently, combining both leaf traits and  $\delta^{15}\text{N}$  values is an  
93 interesting approach to assess ecosystem responses to environmental gradients.

94

95 In this study we address the existing lack of standardized cross-continental research and assessed  
96 shifts in nutrient availability and forest functional composition along two similar transects in  
97 Ecuador and Rwanda. We assessed these shifts through indicative (I) community-level functional  
98 traits and (II) nitrogen isotope ratios in topsoil and canopy. We hypothesized that (I) both these  
99 community-level traits and stable isotope signals would indicate a shift in nitrogen availability  
100 with altitude, and that (II) these shifts would be similar on both continents in terms of direction  
101 and magnitude, given a standardized research protocol and a similar adiabatic lapse rate.

## 102 Materials and Methods

### 103 **Field inventories, sampling and trait analyses**

104 We selected plots at different altitudes on the West flank of the Andes in Ecuador (ranging  
105 from 400-3200 masl) and in the Nyungwe national Park Rwanda (1600-3000 masl), in the  
106 Southern Great Rift Valley (figure S1 and Table S1 for location and overview maps). Due to  
107 reduced accessibility, the gradient in Rwanda was shorter than the South-American transect. We  
108 delineated and inventoried plots following an international standardized protocol for tropical forest

109 inventories (RAINFOR, Malhi et al. 2002), with an adapted plot size of 40 by 40 m. In each plot,  
110 the diameter of all live stems with a diameter larger than 10 cm was measured at 1.3 m height and  
111 the trees were identified to species or genus level. Besides diameters also tree heights were  
112 measured, in order to estimate the aboveground carbon storage (AGC) using pan-tropical  
113 allometric relationships (Chave et al., 2014). The canopy of every plot was characterized by  
114 selecting the most abundant tree species, aiming at a sampling percentage of 80% of the basal area  
115 of the plots. For the selected species of all plots, we sampled mature leaves of a minimum of three  
116 individuals per species per plot using tree climbers. For most of the individuals we sampled fully  
117 sunlit leaves, but this was not always possible for the safety of the climbers, in which case we  
118 sampled partly shaded leaves under the top canopy. Previous work on elevational transects has  
119 shown that the vertical profile of leaves within a canopy has little effect on the trait values (Fisher  
120 et al., 2013). Additionally, composite samples of the topsoil (0-5 cm) were collected at five  
121 different places within each plot, and mixed per plot prior to drying. Soil and leaf samples were  
122 dried for 48 hours at 60°C. Roots were picked out of the soil samples before grinding and  
123 subsequently carbon (C), nitrogen (N) content and  $\delta^{15}\text{N}$  of plant and soil samples were analyzed  
124 using an elemental analyzer (Automated Nitrogen Carbon Analyser; ANCA-SL, SerCon, UK),  
125 interfaced with an Isotope Ratios Mass Spectrometer (IRMS; 20-20, SerCon, UK). Leaf samples  
126 were dry-ashed at 550°C for 5.5 hours; the ash was dissolved in 2M HCl solution and subsequently  
127 filtered through a P-free filter. The aliquots were then analyzed for total P by AAS method No.G-  
128 103-93 Rev.2 (Multitest MT7/MT8; Ryan and others 2001). SLAs were calculated by dividing the  
129 leaf areas of all the sampled leafs per individual by their summed dry mass. Leaf areas were  
130 determined by either photographing leafs with on white paper with a reference scale or by drawing  
131 leaf contours and scanning the drawings. Both the scans and the pictures were processed using the

132 ImageJ software (Schneider et al., 2012). For one abundant species of the higher altitudes on the  
133 Rwandan transect (*Podocarpus latifolius* (Thunb.) R.Br. ex Mirb.) we could not obtain good area  
134 estimates, so we adopted SLA figures from literature (Midgley et al., 1995).

135

### 136 **Statistical analysis**

137 Average leaf trait values as specific leaf area (SLA), leaf nitrogen content on mass basis  
138 (LNC), leaf phosphorus content on mass basis (LPC),  $\delta^{15}\text{N}$ , C:N and N:P ratio were calculated for  
139 every selected species, based on the sample values for the different individuals of the species.  
140 Subsequently, to calculate community-level traits and leaf  $\delta^{15}\text{N}$  per plot, we calculated a basal area  
141 weighted-average canopy value and standard deviation using the species composition and the  
142 species averages, following Asner et al. (2016b). Hence:

$$143 \quad \bar{x}_w = \frac{\sum_{i=1}^N w_i \cdot x_i}{\sum_{i=1}^N x_i}$$

144 with  $x_w$  the weighted value for trait  $x$ ,  $x_i$  the mean trait value for species  $i$  and  $w_i$  the basal-area  
145 based weight of that species in the specific plot. Subsequently for the weighted standard deviations  
146 ( $\sigma_w$ ):

147

148

$$149 \quad \sigma_w = \sqrt{\frac{\sum_{i=1}^N w_i \cdot (x_i - \bar{x}_w)^2}{(N - 1) \sum_{i=1}^N w_i}}$$

150 with  $N$  the number of nonzero weights.

151 The structure of the trait datasets was assessed qualitatively using Pearson correlation statistics  
152 after log-transforming the trait data for normality. Finally, we studied the relations between the

153 different leaf traits and elevation using mixed effects models for the different traits, with a random  
154 error structure. The plots were spatially clustered around four altitudes on both transects, hence we  
155 introduced these elevational clusters as a random effect, and treated altitude and transect as fixed  
156 effects. Models were then fitted using maximum likelihood methods in the ‘lme4’ package in R  
157 (Bates et al., 2007). P-values of the fixed effects –elevation, transect and their interaction - were  
158 determined based on the denominator degrees of freedom calculated with the Satterhwaite  
159 approximation, in the lmerTest package (Kuznetsova et al., 2014). The P-values for the interaction  
160 term, along with the Akaike Information Criterion (AIC) for models with and without this  
161 interaction term were used to decide whether or not to exclude the interaction term. For reasons of  
162 linearity we used the inverse C:N (hence rather N:C) in these analyses. Models for  $\delta^{15}\text{N}$  were  
163 assessed for each transect, using mixed effects models, with elevational cluster as a random effect.  
164 To explicitly determine divergence and convergence of plant and soil  $\delta^{15}\text{N}$  with altitude,  
165 compartment (i.e. canopy leaves or topsoil) was introduced as a fixed effect and the interaction  
166 term was left in the model. For the statistical analysis, the R-software was used (R Core Team,  
167 2014).

168

## 169 Results

170 The pooled trait datasets from both transects showed a consistent and similar correlation structure  
171 (Fig. S2), with both the separate and the pooled data showing significant correlations between all  
172 traits, except SLA and N:P. The structural vegetation parameters on both transects showed  
173 important differences: for the same altitude range, we found a higher stem density, but less species  
174 on the Rwandan transect (Table 1). Tree height and basal area were comparable, and the carbon  
175 stocks showed high variability along both transects. Climatic conditions were similar, with a highly

176 consistent temperature gradient (Fig. S3, Table 1), and similar mean annual precipitation in the  
177 concurring elevational ranges. The linear mixed effects models with altitude as fixed effect, were  
178 able to explain a significant proportion of variation in all traits. This is reflected by both the  
179 marginal (m) and conditional (c)  $R^2_{adj}$ , respectively proxies for the variation explained by the fixed  
180 effects, and the random and fixed effects together (Schielzeth and Nakagawa, 2013) (Table 2). The  
181 interaction term was not significant in any case, hence the trait responses to altitude were parallel  
182 on both continents. LNC, N:C, LPC and N:P significantly decreased with altitude ( $R^2_{adj,m}$  of  
183 respectively 0.83, 0.87, 0.68 and 0.60), with the Rwanda transect showing higher overall values.  
184 SLA also decreased significantly, but with a slightly higher intercept for the Ecuadorian transect  
185 ( $R^2_{adj,m} = 0.83$ ).  $\delta^{15}N$  decreased on both continents with altitude, with a similar effect on both  
186 continents (Table 3). There was a significant divergence between slope and soil  $\delta^{15}N$  along the  
187 Ecuadorian transect, while Rwanda showed a significant convergence ( $R^2_{adj,m} = 0.93$  and 0.55 for  
188 respectively Ecuador and Rwanda).

189

## 190 Discussion

191 Elevational transects are viable setups to assess long-term ecosystem responses to environmental  
192 gradients. We assessed canopy chemistry, functional composition and  $\delta^{15}N$  signals along one  
193 elevational gradient in South-America and one in Central Africa. The measured traits are indicative  
194 proxies for the underlying biogeochemistry of the forest ecosystems. The shifts of these proxies  
195 along both transects were parallel in both setups. They indicated a lowering N availability with  
196 increasing altitude, with a subsequent parallel shift in functional forest composition on both  
197 continents.

198

199 The vegetation structure was varying differently along both transects. The high variability in the  
200 stem number, basal area and carbon stocks is potentially caused by the relatively small plot size.  
201 Other research efforts, targeting these variables in specific, use plot sizes of 1 hectare, as set  
202 forward by the RAINFOR protocol, in tropical forests worldwide (Phillips et al., 2009). As such,  
203 the differing carbon stocks probably do not integrate important stochastic events (e.g. tree fall)  
204 from the forest along both slopes. However, interestingly enough we found a lower average carbon  
205 stocks and higher number of trees in the upper two Rwandan clusters in comparison to the  
206 Ecuadorian forests. This contrasts to what has been reported from large scale forest monitoring  
207 networks across the lowland forests of Amazon and the Congo basin (Lewis et al., 2013). More  
208 research in larger plots, including dynamics and productivity should validate if this is a consistent  
209 observation in highland forest on both continents. On the other hand, the lower species number on  
210 the African transect fits well within the recent findings of a pantropical study, reporting a lower  
211 tree species diversity in the African tropical forest (Slik et al., 2015).

212  
213 Different environmental variables are influenced by altitudinal changes, i.e. atmospheric pressure,  
214 temperature, cloudiness, moisture, etc. (Körner, 2007). ~~Using all these variables in a meta-analysis~~  
215 ~~would be highly interesting, but nonsensical for this study's new data alone, hence we chose to~~  
216 ~~indirectly use~~ Accordingly, elevation ~~as~~ is an indirect proxy for the related changes in these  
217 variables. In this view, the air temperature decrease with elevation was highly similar on both  
218 transects, which means that we can validly assess similar temperature-driven responses of both  
219 forest functional composition and the underlying nutrient dynamics. The high collinearity in the  
220 trait datasets corresponds well to known trade-offs described as the “leaf economics spectrum”  
221 (LES); basically a leaf-level trade-off between leaf construction cost, i.e. low specific leaf area

222 (SLA), leaf nitrogen content (LNC) and leaf phosphorus content (LPC); and photosynthetic  
223 efficiency, i.e. high SLA, LNC and LPC (Wright et al., 2004). LNC, LPC and SLA showed a  
224 highly significant decrease with altitude (Fig. 1 and Table 2), indicating a functional shift towards  
225 more nutrient conservative species communities at higher altitudes on both transects. Indeed,  
226 leaves at lower altitudes with high LNC, LPC and SLA and hence a more efficient photosynthetic  
227 apparatus and rapid turnover, are replaced by leaves with low LNC, LPC and SLA values at higher  
228 altitudes. We've added previous published work of South America and South-East Asia, with  
229 similar temperature gradients by Asner et al, Kitayama and Aiba and Van de Weg et al. to our  
230 transects (Asner et al., 2016b; Kitayama and Aiba, 2002; Van de Weg et al., 2009; Fig. S3) to  
231 assess the consistency of our observed trends. ~~We deliberately only~~We added the limited amount  
232 of studies where community-weighted means were reported along one 'single mountain range  
233 system', hence neglecting a recent and relevant contribution from Asner et al. (Asner and Martin,  
234 2016). ~~As argued by Tanner et al. (Tanner et al., 1998), forests with low, intermediate and high~~  
235 ~~nutrient concentrations can be found globally at any altitude, hence neglecting the effect of local~~  
236 ~~environmental characteristics (atmospheric deposition, seasonality etc.) might lead to wrong~~  
237 ~~conclusions. This is also shown by the different intercepts, or the vertical shift in trends in Fig. S3.~~  
238 Our comparison showed that the decreasing trend in LNC was consistent with the other studies  
239 from South-America (Asner et al., 2016b; Van de Weg et al., 2009), but not with South-East Asia  
240 where no significant trend was found (Kitayama and Aiba, 2002). However, leaf mass area (LMA;  
241 the inverse of SLA) of all studies showed a similar, increasing trend with elevation. LPC shows a  
242 strong and significant trend along both transects in this study, while the other studies report no  
243 significant trend. This is consistent with the meta-analysis presented by Tanner et al., which shows  
244 consistent negative LNC trends on 'same mountain' studies and inconsistent LPC trends (Tanner

245 et al., 1998). A recent effort on a larger scale in Peru has shown that LES trade-off between LNC-  
246 LPC or SLA-LPC is indeed decoupled by climatic and geophysical filters, while the leaf SLA-  
247 LNC trade-off is more robust (Asner et al., 2016a). Regarding the studies we included for  
248 comparison (Fig. S3), only Van de Weg et al. assessed N:P ratio, ~~and although~~. Although no  
249 significant trend was found, they reported that N:P ratio was lowest in the highest sites (Van de  
250 Weg et al., 2009). Additionally, decreasing N:P ratios have also been reported on other transects  
251 on the Andes (Fisher et al., 2013; Soethe et al., 2008), and recently in Peru using airborne imaging  
252 spectroscopy (Asner et al., 2016a).

253

254 In addition to the above community-level functional traits, the decreasing  $\delta^{15}\text{N}$  values on both  
255 continents (Fig. 1) are another strong indication of the decreasing N availability in the upper  
256 forests. Along the transects, both topsoil and canopy leaves showed decreasing  $\delta^{15}\text{N}$  values with  
257 increasing altitude (Fig. 1), indicating a more closed N-cycle with lower N availability at the  
258 higher altitudes of both transects. It has been shown that lowland tropical rainforests exhibit high  
259 values of  $\delta^{15}\text{N}$  mainly caused by the high gaseous nitrogen losses via denitrification, a strongly  
260 fractionating process (Houlton et al., 2006). The decreasing trends with altitude are interesting and  
261 seem to support the existing paradigm that tropical forests shift from P to N limitation in transition  
262 from lowland to montane tropical forest (Townsend et al., 2008). This is also reflected in the  
263 stoichiometric shifts, as canopy N:P is decreasing with increasing elevation (Fig. 1). Hence plants  
264 incorporate relatively less N compared to P in canopies at higher altitudes. The higher soil  $\delta^{15}\text{N}$   
265 values along the lower part of the Rwanda transect suggests a more open N-cycle compared to the  
266 lower part of the Ecuadorian transect. This corroborates with a recent finding of very high N losses  
267 at 1900 masl at the Rwanda site (Rütting et al., 2014), and the observation of high retention

268 potential of bio-available N in Chilean Andisols (Huysens et al., 2008). Further research is needed  
269 to explain the notable divergence in soil and foliage  $\delta^{15}\text{N}$  along the Ecuadorian transect, mainly  
270 driven by the highest elevational cluster. As previously reported this can be due to different degrees  
271 of dependence upon ectomycorrhizal fungi (EcM) (Hobbie et al., 2005), different mycorrhizal  
272 association types (Craine et al., 2009) or shifts in the uptake of different forms of nitrogen (Averill  
273 and Finzi, 2011; Kahmen et al., 2008). EcM-associated plant species are expected to show more  
274 depleted isotopic ratios, due to isotope fractionation during N transfer to the host plant. This effect  
275 is obscured in lowland N-rich tropical forests and might just not be detectable at lower altitudes,  
276 but might become apparent in N-poorer environments such as the higher altitude forests (Mayor  
277 et al., 2014). Secondly, a study from a temperate elevational transect has shown that plants  
278 increasingly switch to organic N sources with decreasing temperature, without fractionation upon  
279 N transfer from EcM to plants (Averill and Finzi, 2011). Resulting from that, they found a  
280 convergence rather than divergence of  $\delta^{15}\text{N}$  soil – canopy along altitude, because plants draw N  
281 increasingly from a source pool close to the bulk isotopic signature. We have no data on EcM  
282 colonization or  $\delta^{15}\text{N}$  of sporocarps in the study plots, so we are not able to disentangle both  
283 mechanisms. However, by characterizing both community functional traits and canopy and soil  
284  $\delta^{15}\text{N}$ , the data of these transects is consistent with a decreasing availability of soil N as elevation  
285 increases. We suggest the reduced N availability to be caused by an indirect temperature effect on  
286 the N-cycle, consistent with observations from a direct fertilization experiment (Fisher et al.,  
287 2013). Lower temperatures slow down depolymerization and N mineralization processes, hence  
288 also N bio-availability, thereby invoking changes in the functional plant communities along the  
289 transects (Coûteaux et al., 2002; Marrs et al., 1988). Future global change will most likely distort  
290 N availability both directly via increased reactive N deposition (Galloway et al., 2008; Hietz et al.,

291 2011) and indirectly via a temperature effect on N mineralization in forest soils. This raises  
292 questions on the future of plant species within the already threatened montane tropical forest  
293 biome, where higher N availability and temperature increase might distort the existing ecological  
294 niches and in turn also increase N-losses. Further research should therefore focus on process-  
295 based knowledge of N and P cycle dynamics along such transects to further assess if the availability  
296 is actually limiting the ecosystems. These observations also have repercussions for carbon fluxes:  
297 since nutrient availability exerts a stronger control on NEP than on gross primary production (GPP)  
298 (~~Fernández-Martínez~~Fernandez-Martinez et al., 2014), it is likely that the CUE<sub>e</sub> will be lower at  
299 higher altitudes. It has been hypothesized that this decrease in CUE<sub>e</sub> is due to an increased  
300 investment of photosynthates to non-biomass components, such as root symbionts for nutrient  
301 mining and root exudates, in expense of net primary production (NPP) (Vicca et al., 2012).  
302 However, recent empirical evidence has shown for one transect in the Andes, that a decrease in  
303 GPP with increasing altitude is not accompanied by a trend in CUE (Malhi et al., 2016). More  
304 work on carbon budgets along elevational transects is needed to fully understand the role of N and  
305 P availability and its interaction with climate gradients for the tropical forest carbon cycle.

306

## 307 **Conclusions**

308 Altogether, this study evidences parallel functional shifts with a similar direction and magnitude  
309 along two comparable elevation gradients, in tropical forests on two different continents. The data  
310 suggests, in two different ways, that this shift is caused by temperature-driven response of nutrient  
311 availability. With the first data on an elevational transect in Central Africa, this work adds to the  
312 existing set of elevational transects in the tropics. However, more transects are needed, especially  
313 in Africa, to validate a universal response of tropical forests to environmental change.

314 Furthermore, work on process-based nutrient dynamics is important to unravel the importance of  
315 different global change factors for both forest basins.

316

## 317 **Supplementary information**

318 **Fig. S1** Overview map

319 **Fig. S2** Structure and correlations of the trait data

320 **Fig. S3** Trends in community-level functional traits of previously reported studies

321 **Table S1** Coordinates, elevation and cluster membership of the different plots on both transects

322 **Table S2** Summary of the plot-level characteristics

323 **Table S3** Fixed effects estimates for the different canopy-level response variables for the full  
324 model including interaction term

325

## 326 **Author contributions**

327 *M.B., H.V. and P.B. developed the project; M.B, M.D., S.B., C.T. and D.V. carried out the field*  
328 *work and analyzed the data. All authors contributed to the ideas presented and edited the*  
329 *manuscript.*

330

## 331 **Competing interests**

332 The authors declare that they have no conflict of interest.

333

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556 **Table 1** General characteristics, vegetation structure, climate (mean annual temperature (MAT) and mean annual precipitation (MAT),  
557 WorldClim - Fick, 2017) and soil characteristics of the elevational clusters on both transects. Number of trees and species (in the 40  
558 by 40 m plots), basal area (BA), mean tree height (MTH) and above-ground carbon (AGC) are averages per plot  $\pm$  the standard  
559 deviation on the plot-level results, based on the inventories.

|         | Cluster | Altitude (masl) | Number of trees per plot | Number of species per plot | BA (m <sup>2</sup> ha <sup>-1</sup> ) | MTH (m)        | AGC (Ton C ha <sup>-1</sup> ) | MAT (°C) | MAP (mm) | Soil parent material   | Soil classification |
|---------|---------|-----------------|--------------------------|----------------------------|---------------------------------------|----------------|-------------------------------|----------|----------|------------------------|---------------------|
| Ecuador | 1       | 406 $\pm$ 10    | 86 $\pm$ 13              | 30 $\pm$ 2                 | 25 $\pm$ 3                            | 18.3 $\pm$ 1.0 | 96 $\pm$ 19                   | 23.7     | 3720     | Lahars                 | Andisol             |
|         | 2       | 1068 $\pm$ 25   | 84 $\pm$ 38              | 39 $\pm$ 15                | 33 $\pm$ 9                            | 16.9 $\pm$ 0.7 | 140 $\pm$ 25                  | 20.0     | 3227     | Lahars                 | Andisol             |
|         | 3       | 1871 $\pm$ 79   | 69 $\pm$ 11              | 31 $\pm$ 2                 | 33 $\pm$ 11                           | 13.5 $\pm$ 1.3 | 112 $\pm$ 57                  | 17.5     | 1619     | Redbed volcaniclastics | Andisol             |
|         | 4       | 3217 $\pm$ 21   | 90 $\pm$ 25              | 18 $\pm$ 2                 | 49 $\pm$ 11                           | 13.0 $\pm$ 1.4 | 161 $\pm$ 34                  | 10.9     | 1241     | Granitic/acid          | Andisol             |
| Rwanda  | 1       | 1760 $\pm$ 66   | 70 $\pm$ 18              | 21 $\pm$ 4                 | 34 $\pm$ 4                            | 13.9 $\pm$ 0.7 | 121 $\pm$ 11                  | 17.6     | 1518     | Shale and Quartzite    | Inceptisol/Ultisol  |
|         | 2       | 2200 $\pm$ 64   | 71 $\pm$ 18              | 18 $\pm$ 3                 | 45 $\pm$ 9                            | 14.3 $\pm$ 0.3 | 179 $\pm$ 27                  | 15.9     | 1628     | Shale and Quartzite    | Inceptisol/Ultisol  |
|         | 3       | 2512 $\pm$ 37   | 122 $\pm$ 60             | 11 $\pm$ 1                 | 31 $\pm$ 9                            | 12.0 $\pm$ 1.0 | 99 $\pm$ 36                   | 14.7     | 1716     | Shale and Quartzite    | Inceptisol/Ultisol  |
|         | 4       | 2844 $\pm$ 77   | 109 $\pm$ 56             | 8 $\pm$ 2                  | 34 $\pm$ 4                            | 11.5 $\pm$ 0.5 | 89 $\pm$ 10                   | 12.9     | 1835     | Shale and Quartzite    | Inceptisol/Entisol  |

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562 **Table 2** Fixed effects estimates (altitude in km asl) for the different canopy-level response  
 563 variables; leaf nitrogen content (LNC), inverse C:N ratio, specific leaf area (SLA), leaf phosphorus  
 564 content (LPC) and N:P ratio, along with the estimated marginal (m) and conditional (c)  $R^2_{adj}$  (sensu  
 565 Nakagawa and Schielzeth (5)). The interaction term for altitude x transect was not significant in  
 566 any case, and was hence not retained in any model.

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| Response                            | Effect            | Estimate | SE     | P-value | $R^2_{adj,m}$ | $R^2_{adj,c}$ |
|-------------------------------------|-------------------|----------|--------|---------|---------------|---------------|
| LNC (%)                             | Ecuador intercept | 3.04     | 0.131  | <0.001  | 0.80          | 0.85          |
|                                     | Rwanda intercept  | 3.65     | 0.115  | 0.003   |               |               |
|                                     | Altitude          | -0.59    | 0.000  | <0.001  |               |               |
| SLA ( $\text{cm}^2 \text{g}^{-1}$ ) | Ecuador intercept | 175.28   | 14.107 | <0.001  | 0.77          | 0.95          |
|                                     | Rwanda intercept  | 172.55   | 12.504 | 0.835   |               |               |
|                                     | Altitude          | 36.24    | 0.007  | 0.002   |               |               |
| N:C                                 | Ecuador intercept | 0.07     | 0.003  | <0.001  | 0.83          | 0.91          |
|                                     | Rwanda intercept  | 0.07     | 0.003  | 0.033   |               |               |
|                                     | Altitude          | -0.01    | 0.000  | <0.001  |               |               |
| LPC (%)                             | Ecuador intercept | 0.16     | 0.012  | <0.001  | 0.60          | 0.88          |
|                                     | Rwanda intercept  | 0.17     | 0.011  | 0.247   |               |               |
|                                     | Altitude          | -0.02    | 0.000  | 0.009   |               |               |
| N:P                                 | Ecuador intercept | 20.66    | 1.055  | <0.001  | 0.54          | 0.74          |
|                                     | Rwanda intercept  | 24.06    | 0.926  | 0.014   |               |               |
|                                     | Altitude          | -1.82    | 0.001  | 0.018   |               |               |

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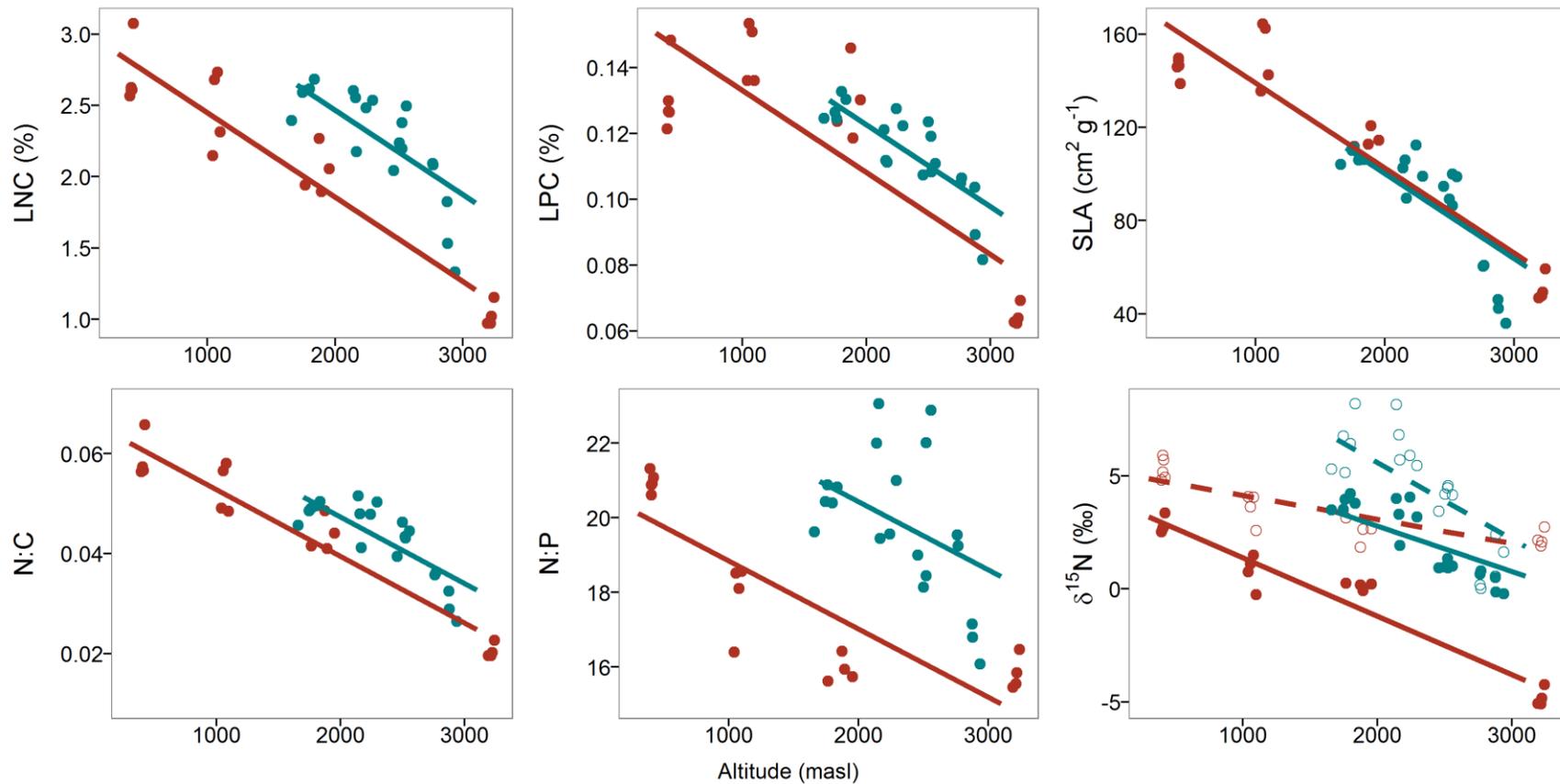
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572 **Table 3** Fixed effects estimates (altitude in km asl) for  $\delta^{15}\text{N}$  in both canopy and topsoil  
 573 (compartment) on both transects, along with the estimated marginal (m) and conditional (c)  $R^2_{\text{adj}}$   
 574 (sensu Nakagawa and Schielzeth (5)).

| Response                         | Effect                 | Estimate | SE    | P-value | $R^2_{\text{adj,m}}$ | $R^2_{\text{adj,c}}$ |
|----------------------------------|------------------------|----------|-------|---------|----------------------|----------------------|
| $\delta^{15}\text{N}$ Ecuador(‰) | Canopy intercept       | 3.96     | 0.406 | <0.001  | 0.93                 | 0.94                 |
|                                  | Soil intercept         | 5.19     | 0.440 | 0.009   |                      |                      |
|                                  | Altitude               | -2.59    | 0.000 | <0.001  |                      |                      |
|                                  | Altitude x compartment | 1.53     | 0.000 | <0.001  |                      |                      |
| $\delta^{15}\text{N}$ Rwanda (‰) | Canopy intercept       | 6.83     | 3.193 | 0.21103 | 0.55                 | 0.82                 |
|                                  | Soil intercept         | 12.37    | 1.749 | 0.004   |                      |                      |
|                                  | Altitude               | -2.02    | 0.001 | 0.310   |                      |                      |
|                                  | Altitude x compartment | -1.37    | 0.001 | 0.074   |                      |                      |

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577 **Fig. 1. Trends in community-level functional traits and leaf (full line, closed circles) and topsoil (dashed line, open circles)  $\delta^{15}\text{N}$**   
 578 **of the elevation transects in Ecuador (red) and Rwanda (blue). Leaf nitrogen content (LNC), leaf phosphorus content (LPC), specific**  
 579 **leaf area (SLA), and leaf N:C, P:C and N:P ratio decrease with increasing altitude on both transects. Both transect showed decreasing**

580 values of  $\delta^{15}\text{N}$ , providing additional evidence for a more closed N-cycle with increasing altitude. Lines represent the fixed altitude  
581 effects in the respective statistical models for both Ecuador (red, 400-3200 masl) and Rwanda (blue, 1600-3000 masl).  
582