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Sellan, Giacomo , Thompson, Jill , Majalap, Noreen and Brearley, Francis Q  (2022) Influence of species functional strategy on leaf stoichiometric responses to fertilizer in a Bornean heath forest. *Journal of Ecology*, 110 (6). pp. 1247-1258. ISSN 0022-0477

**DOI:** <https://doi.org/10.1111/1365-2745.13865>

**Publisher:** Wiley

**Version:** Accepted Version

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**Data Access Statement:** The data are freely available on the Natural Environment Research Council (NERC) Environmental Information Data Centre (<https://doi.org/10.5285/d5ea9e2c-f053-4631-aead-a8a5557488c6>).

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## Influence of species functional strategy, on leaf stoichiometric responses to fertilizer in a Bornean heath forest

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**Abstract:**

- 1) The distribution of Bornean heath forest on white sand soils is believed to be due to element limitation and soil acidity.
- 2) To determine the impact of both element limitation and soil acidity on tropical heath forest we established a soil fertilization experiment to investigate the impact that increased soil N availability and reduced soil pH (using lime/CaCO<sub>3</sub>) had on a range of elements in tree leaves. We hypothesized that alterations in soil resource availability would cause changes in the tree leaf N:P ratio and concentrations of other elements and these changes would be influenced by species' functional strategies.
- 3) The experiment was carried out in a Bornean heath forest on infertile soil over a two-year period. We selected ten common tree species, spanning acquisitive to conservative strategies, and tested whether tree species functional strategies influenced tree leaf elemental concentrations after the fertilization.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/1365-2745.13865](https://doi.org/10.1111/1365-2745.13865)

- 4) Leaf N:P ratios showed considerable differences among co-occurring species. Overall, we found that soil N addition treatments increased leaf N concentration, although leaf N:P ratios were not affected. Changes in leaf Al, Fe and S concentrations were correlated with species functional strategy: conservative species showed a greater increase in leaf Fe compared with acquisitive species, whereas acquisitive species showed a greater increase in leaf Al but a decrease in leaf S compared with conservative species.
- 5) **Synthesis.** We show that soil elements uptake differs between acquisitive and conservative species and that acquisitive species may not take up soil elemental resources more effectively than conservative species. We suggest that the greater Fe acquisition by conservative species, in comparison to acquisitive species, might be due to a stress tolerance strategy. The overall increase in leaf N showed that, in this nutrient poor forest, N is a fundamental requirement irrespective of species functional strategies. Given the increased leaf Al and Fe concentration after liming, we also suggest that these elements are important, and possibly limiting (Fe), in this heath forest.

### **Abstract in bahasa Melayu;**

- 1) Taburan ketumbuhan hutan kerangas di Borneo atas tanah jenis pasir putih dipercayai disebabkan oleh had pada unsur dan keasidan tanah.
- 2) Untuk mengetahui apakah impak kepada hutan kerangas tropika sekiranya terjadi pengehadan dari segi unsur berserta keasidan tanah, kami telah menjalankan satu kajian pembajaan tanah untuk menyiasat kesan peningkatan dalam ketersediaan N

tanah bersama penurunan pH tanah (menggunakan kapur/ $\text{CaCO}_3$ ) terhadap tahap beberapa jenis unsur dalam daun pokok. Kami berdasarkan hipotesis bahawa perubahan dalam ketersediaan bekalan tanah akan mengakibatkan perubahan pada nisbah N:P dalam daun pokok dan kepekatan unsur-unsur lain yang mana perubahan-perubahan ini akan dipengaruhi oleh strategi-strategi fungsi mengikut spesies.

- 3) Kajian telah dijalankan selama dua tahun di satu hutan kerangas di Borneo dengan tanah yang tidak subur. Dengan memilih sepuluh spesies kayu yang biasa terdapat di situ serta mewakili yang bersifat berstrategi akuisitif dan yang berstrategi konservatif, kami telah menguji samada strategi fungsi spesies pokok mempengaruhi tahap kepekatan unsur dalam daun pokok selepas pembajaan.
- 4) Nisbah pada N:P daun memperlihatkan perbezaan ketara diantara spesies yang tumbuh bersama. Secara keseluruhan, kami mendapati rawatan penambahan pada N tanah menyebabkan peningkatan pada kepekatan N dalam daun tetapi tidak membawa kesan kepada nisbah N:P daun. Perubahan pada kepekatan Al, Fe dan S daun didapati berkait dengan strategi fungsi spesies: spesies bersifat konservatif menunjukkan peningkatan lebih besar pada Fe daun berbanding dengan spesies bersifat akuisitif manakala spesies akuisitif pula menunjukkan peningkatan lebih tinggi pada Al daun tetapi penurunan pada S daun berbanding spesies konservatif.
- 5) **Sintesis.** Kami menunjukkan bahawa pemerapan unsur-unsur tanah berbeza antara spesies bersifat akuisitif dan yang bersifat konservatif, dan kemungkinannya spesies akuisitif tidaklah memerap bekalan unsur tanah lebih cepat dari spesies konservatif. Kami mencadangkan bahawa pemerapan bagi Fe yang lebih tinggi pada spesies konservatif mungkin disebabkan oleh strategi toleransi terhadap tekanan.

Peningkatan keseluruhan pada N daun menunjukkan bahawa, bagi hutan miskin nutrien ini, N adalah keperluan asas tanpa mengira strategi fungsi spesies. Melihat peningkatan pada kepekatan Al dan Fe dalam daun selepas penambahan kapur, kami juga mencadangkan bahawa unsur-unsur tersebut, dan mungkin juga pengehadan (Fe), adalah penting dalam hutan kerangas ini.

**Keywords:** functional gradient; kerangas; leaf stoichiometry; liming; N fertilization; rain forest nutrient limitation; white-sand forest.

# 1 Introduction

Terrestrial ecosystem productivity is often considered to be limited by two key soil elements, nitrogen (N) and phosphorus (P; Elser et al. 2007), and N deposition from pollution is increasingly disrupting soils' and plant communities' equilibrium (Phoenix et al. 2006). Tropical heath forest is important for biodiversity and is a rare ecosystem in need of conservation. In Borneo, heath forest is under increasing threat from nitrogen pollution, thus an assessment of element limitation is fundamental for predicting its future, and the potential impact of increasing atmospheric element deposition on heath forest species composition (Bobbink et al. 2010). Soil nitrogen (N) is mainly derived from biological fixation by heterotrophic microorganisms that find optimal conditions at tropical latitudes (Hedin et al. 2009). Soil phosphorous (P) becomes available to plants through mineral dissolution and is quickly lost through weathering and leaching (Porder et al. 2007). These different processes for N and P suggest that in younger temperate soils, plant productivity is limited by N, whereas in older weathered tropical soils productivity is limited by P (Vitousek 1984; Vitousek & Howarth 1991). However, recent meta-analyses of fertilization experiments (Wright et al. 2018; Wright 2019) propose N and P are co-limiting in lowland tropical rain forests.

Both soil and leaf N and P concentrations generally increase from tropical to temperate latitudes whereas leaf N:P ratios decrease along the same gradient (Hedin 2004; Reich & Oleksyn 2004). Leaf N:P ratios have often been used as a proxy to assess soil element limitation and to characterise biogeochemical cycles (Koerselman & Meuleman 1996; Güsewell 2004; Townsend et al. 2007). Soil-leaf N and P relationships are not straightforward in the tropics, as, for example, leaf N:P ratios vary greatly among species (Güsewell 2004), and at small spatial and temporal scales (Sullivan et al. 2014).

We propose that the relationships among leaf N:P ratios and soil element limitation (Ostertag & Dimanno 2016) may be affected by species functional strategy. There are many gradients and definitions that identify species functional strategies; in this study we adopt the continuum of variation from “acquisitive” to “conservative” strategies (Reich 2014). Acquisitive species typically have low wood density and high leaf element concentrations, they are believed to rapidly acquire available resources and grow fast, but are short lived (van der Sande et al. 2018). The rapid acquisition of available resources suggests they may increase their leaf element concentrations and alter their leaf N:P ratio after soil N addition more quickly than conservative species. Conservative species, on the other hand, are comparatively long lived, may be shade- or drought-tolerant and have slower leaf turnover (Chapin 1980; Wright et al. 2004; van der Sande et al. 2018), so that changes in leaf element concentrations, after soil fertilization, might only be measurable after a longer period of time. Functional traits have been shown to predict species’ growth responses to fertilization experiments in Ecuadorian montane forest (Baez & Homeier 2018), and community compositional shifts in temperate grasslands (Dickson et al. 2014). We do not believe that any study has investigated the effect of alterations to soil element resource availability on leaf elemental concentrations among trees species with contrasting functional strategies.

In addition to N and P, other elements have important ecophysiological functions and might co-limit plant growth if in short supply (Kaspari & Powers, 2016). For example iron (Fe) and copper (Cu) are necessary enzymatic components that detoxify superoxide radicals; calcium (Ca) is fundamental for signal conduction and cell structure, and magnesium (Mg) for the regulation of cellular pH as well as cation-anion balance (Marschner 1995). Most of these macro- and micro-elements show a broader global variation in leaves than N and P (Metali et al. 2012). Such variation is primarily due to environmental factors but also to genetic factors (Watanabe et al. 2007). Plant uptake of soil elements is also regulated by the co-uptake of metals in the soil, which can be toxic

in high amounts (Foy et al. 1978, Trethowan et al. 2021). Plants, for example, can either limit the uptake of an element, if this requires the co-uptake of a toxic metal, or they can store the excess toxic metal in non-metabolically important organs, such as the vacuole.

Soil pH is an environmental factor that plays a key role in leaf elemental concentrations by influencing the availability of elements in soil through soil mineralisation and soil organic matter decomposition (Chapin et al. 2002). A pH close to neutral generally increases soil cation exchange capacity, which allows plants to absorb, store and invest in useful elements (Aluko 1990; Wright et al. 1991). The relationships between soil pH and tropical forest tree leaf elements requires investigation, and we are not aware of studies that have investigated tropical forest tree stoichiometric responses to experimental soil pH manipulations.

Here we focussed on a tropical heath forest in Borneo, this forest type is found throughout the tropics and is characterised by a unique tree species composition (Newbery 1991, Fine et al. 2010, Oktavia et al. 2021). Trees in heath forests have low productivity (Miyamoto et al. 2007, 2016), and leaves with low element concentrations (Turner et al. 2000; Luizão et al. 2004; Peace & Macdonald 1981). The stunted tree characteristics of the heath forests in Borneo have been proposed to be due to acidic soil pH (Proctor 1999; Luizão et al. 2007), low N availability (Proctor et al. 1983; Luizão 1994; Proctor 1999; Moran et al. 2000; Brearley et al. 2011) or to an interaction between the two (Luizão 1996). More recently, co-limitation by both N and P was proposed for tropical heath forests (Dent et al. 2006), and some evidence has been collected for Fe limitation of heath forest soil bacterial communities (Kerfahi et al. 2018).

In this study, we analysed the impact of soil N fertilization and changes in soil pH (with  $\text{CaCO}_3$  addition) on the elemental concentrations in leaves from trees of ten common species. We added  $\text{CaCO}_3$  to the soil aiming to increase soil pH and thus increase element availability. Our hypotheses



were that a) N fertilization and reductions in soil acidity will affect the element concentrations of tree leaves in this unfertile forest, and that b) species' responses are mediated by their functional strategy. We tested these hypotheses by studying the change in leaf element concentrations in response to a fertilization experiment, and investigated the relationship between these changes in element concentrations and species' functional strategy. Understanding these aspects of tropical heath forests' ecology will allow predictions of changes in biodiversity of this rare forest type under global changes, such as increasing N pollution.

## 2 Methods

### 2.1 Study site and experimental design:

We carried out this study in the Kabili-Sepilok Forest Reserve, Sabah, Malaysian Borneo (5° 51'N, 117° 58'E). The forest reserve hosts four forest typologies, but we focussed on the heath forest in the eastern side of the reserve. The climate is equatorial, with an average annual temperature of 26 °C and precipitation of ~3000 mm per year. Only in the dry season (approximately March to May) does the precipitation drop below 100 mm per month (Fox 1973, Nilus 2003). The soil underlying this heath forest area is an infertile spodosol (Sellan et al. 2019) with low concentrations of available nitrogen ( $8.5 \pm 1.1 \mu\text{g NH}_4^+ \text{g}^{-1}$  and  $0.59 \pm 0.1 \mu\text{g NO}_3^- \text{g}^{-1}$  using 1M KCl extraction), and extractable phosphorus ( $14.1 \pm 1.2 \mu\text{g g}^{-1}$  using Mehlich I extraction) and acidic pH ( $3.71 \pm 0.04$ ) (for details see Sellan et al. 2019).

The fertilization experiment was established in 2016 by Sellan et al. (2020), and consisted of 16 plots of 225 m<sup>2</sup> (15 m x 15 m). In July 2016 we started a factorial experiment consisting of four treatments including a control (with no fertilization), addition of N as urea alone, addition of CaCO<sub>3</sub> alone, or N and CaCO<sub>3</sub> together, with four replicate plots for each treatment. We applied fertilizer approximately every six months between July 2016 and February 2018. The same amount of

nitrogen was added at each application at a rate of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The amount of CaCO<sub>3</sub> applied was changed at each fertilizer application to avoid raising soil pH above that of the surrounding lowland evergreen rain forest (pH ~5; Sellan et al. 2021) the first year had 5.56 Mg ha yr<sup>-1</sup> with subsequent years having 1.11 Mg ha yr<sup>-1</sup>. To estimate the amount of CaCO<sub>3</sub> to be used, we measured soil pH before each application of the chemicals. For a detailed description of the experimental design see Sellan et al. (2020).

We identified the ten most common tree species throughout the experimental plots, which represented 57 % of the total number of stems ≥ 1 cm DBH (diameter at breast height, 1.3 m from the ground) and 49 % of the total basal area. The ten species were: *Actinodaphne borneensis* (Lauraceae), *Chionanthus pluriflorus* (Oleaceae), *Cotylelobium melanoxylon* (Dipterocarpaceae), *Diospyros fusiformis* (Ebenaceae), *Dracaena elliptica* (Asparagaceae), *Gaertnera junghuhniana* (Rubiaceae), *Pimelodendron griffithianum* (Euphorbiaceae), *Syzygium* sp. (Myrtaceae), *Ternstroemia aneura* (Pentaphyllacaceae) and *Tristaniopsis obovata* (Myrtaceae). In every experimental plot, we collected around ten fresh leaves from one individual of each of the target species that were present in that plot, although not every species was present in all plots (Supplementary Table S1). Samples were collected and exported in agreement with the Sabah Biodiversity Council, licence number JKM/MBS.1000-2/2 JLD.4 (54) and JKM/MBS.1000-2/3 JLD.3 (11). We collected leaves once before the fertilization treatments started (April 2016), and again before each of the fertilizer applications in February 2017, July 2017 and in July 2018 (we did not collect the leaves before the fertilizer application in January 2018). The same individual trees were sampled at each leaf collection, but if a tree previously sampled had died, we sampled leaves from another tree of the same species in the same plot. Mature leaves from the tree peripheral canopy were collected with a pruner at a height of ~ 7 m. The canopy of heath forests is quite open, so the

leaves were not in full shade. After each leaf collection, we oven dried the fresh leaves at 50 °C and ground the leaves from each individual tree together before chemical analysis. Using leaves from the final leaf collection (July 2018) we measured their specific leaf area (SLA) as the quotient of fresh leaf area and leaf dry mass.

## 2.2 Quantification of variables

We determined foliar Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, P, S and Zn by digesting 0.25 g of ground leaf material with a mixture of 8 ml HNO<sub>3</sub> + 2 ml of deionised H<sub>2</sub>O using a microwave (Mars Xpress 5, CEM Corporation). After dilution in deionised water, we measured the elemental concentration with an inductively coupled plasma-atomic emission spectrometer (iCAP 6300, Thermo Scientific). To measure C and N concentration, we analysed 0.15 g of ground leaves with a Leco TruSpec CN elemental analyser.

We measured functional traits from leaf samples collected from trees in the control plots including specific leaf area (SLA), tree wood density and stem relative growth rate. We considered only individuals from control plots to avoid measurement biases due to the fertilization treatments. We define functional traits as morphological, physiological or phenological characters of species that indirectly affect species' reproductive success or fitness (Violle et al. 2007). Tree wood density values were obtained from the literature (i.e. Zanne et al. 2009; Carsan et al. 2012); when wood density values for the target species were not available, we averaged the available data for congeneric species from the same geographic area as suggested by Flores and Coomes (2010) and Jucker et al. (2016). We measured the DBH of all trees from the selected species in the control plots at the start of the experiment (2016) and after two years (2018). We calculated the relative growth rate from annual stem diameter growth rates using the following formula:  $((d_2 - d_1) / d_1) * 1 / \Delta t$  where  $d_1$  is the initial diameter,  $d_2$  is the diameter at the end of the second year, and  $\Delta t$  is the time interval between the two measurements expressed in years.

### 2.3 Statistical analysis:

We conducted the statistical analyses with R 3.6.2 (R Development Core Team 2019). To study the effect of the fertilizer treatments on leaf stoichiometry we calculated the difference in leaf element concentrations, averaged by plot for all ten target species pooled together, between the first and the last leaf sampling. We expressed this number as the change in leaf elemental concentrations, and we assessed its significance across treatments with an ANOVA followed by Tukey's tests.

We identified species' functional strategies by performing a phylogenetically corrected principal components analysis (PCA) using the *phyl.pca()* function from the "phytools" R package (version 0.6-99; Revell 2009, 2012). For this PCA, we analysed the species' averaged, centred and scaled values including the three functional traits described above. We used a phylogenetically corrected PCA because it accounts for the non-independence of trait measurements taken from species of the same family or the same or higher taxa. The phylogenetic data was derived from a plant family resolved super-tree provided by Zanne et al. (2014), pruned to consist of only our ten target species using the *Phyloomatic* utility (Webb and Donoghue 2005). We did compare the phylogenetically controlled vs. the non-phylogenetically controlled PCA, but there were no major changes on the scores for species on the first or second axes, so we used the results from the phylogenetically controlled PCA. Species' scores were extracted from the first two PCA axes, and the influence of functional strategy on tree species' change in leaf elemental concentration after fertilization treatments was assessed using linear mixed models (R package "lme4" version 1.1-21. Bates et al. 2020). We built linear mixed models (LMM) including treatments (described as two distinct categorical variables with two levels: presence or absence of the N and CaCO<sub>3</sub> treatment; the N + CaCO<sub>3</sub> treatment is thus described by the interaction of the N and the CaCO<sub>3</sub> treatments) and each of the first two PCA axes, including the interaction between treatments and PCA axes.

We also included a model without the PCA axes scores as a null model, and compared the models through a likelihood ratio test (Nickerson 2000). The models were as follows:

a) Variable =  $aN + bCaCO_3 + c \times N \times CaCO_3 + dZ_1 + E$ ,

b) Variable =  $aN + bCaCO_3 + c \times N \times CaCO_3 + dZ_2 + E$ ,

c) Variable =  $aN + bCaCO_3 + cZ_1 + d \times N \times CaCO_3 + e \times N \times Z_1 + f \times CaCO_3 \times Z_1 + g \times N \times CaCO_3 \times Z_1 + E$ ,

d) Variable =  $aN + bCaCO_3 + cZ_2 + d \times N \times CaCO_3 + e \times N \times Z_2 + f \times CaCO_3 \times Z_2 + g \times N \times CaCO_3 \times Z_2 + E$ ,

e) Variable =  $aN + bCaCO_3 + c \times N \times CaCO_3 + E$ .

In these models,  $Z_1$  represents PCA axis 1 (which is characterised by a SLA gradient) and  $Z_2$  represents PCA axis 2 (which is characterised by a wood density and growth rate gradient). The E term included plot (four replicates per treatment) and month of sample collection (four collections) as random effects to avoid pseudo-replication and temporal autocorrelation (Millar and Anderson, 2007). We did not include individuals as a random effect because this would lead to overly complex random effects structure. After running the models, we selected the best model based on the lowest Akaike Information Criteria (AIC), and assessed significance for each single level of the fixed effect using the *lmerTest* R package (version 3.1-1; Kuznetsova et al., 2017). This package calculates pairwise t-tests among levels of the fixed effect and derives the p-values using the Satterthwaite's approximation to estimate degrees of freedom, which is a conservative method to obtain t-tests' p-values (Kuznetsova et al., 2017). We also calculated the conditional and marginal  $r^2$  with the *performance* R package (version 0.4.6; Lüdtke et al. 2020). When a multiplicative interaction of any treatment with the first or second PCA axis significantly influenced leaf element concentration, we calculated a Pearson correlation coefficient between the predicted

change in leaf element concentration by species, and used the loadings of each species on the appropriate PCA axis.

Linear mixed models were used to predict the changes in leaf element concentrations among treatments, where the model structure allowed us to specifically predict the effect of fertilization across species, compared to the control treatment. We used predictions because they are more reliable than the parameters obtained from the linear mixed model's fixed effects only. This model included species, and the multiplicative interaction of species with fertilizer treatment (both were categorical variables with ten levels in the case of species, and four levels in the case of treatments) as fixed effects. We tested the models against corresponding null models using likelihood ratio tests (Bates et al. 2015). Predicted means and intervals were calculated from 1000 simulations using the *merTools* R package (version 0.5.2; Knowles et al. 2020).

### 3 Results

#### 3.1 Leaf stoichiometry and its overall response to the fertilizer addition treatments:

Before the fertilizer treatments the ten target tree species in our study site had a mean leaf N concentration of  $12.60 (\pm 0.09 \text{ SE}) \text{ mg g}^{-1}$ , P concentration of  $0.44 (\pm 0.03) \text{ mg g}^{-1}$  and a mean N:P ratio of  $28.68 (\pm 0.97)$  (Supplementary Table 2). Overall, after two years of fertilizer addition, the addition of N to the soil significantly increased leaf N and C concentrations (Figure 1). None of the treatments influenced the overall leaf N:P ratio (mean of  $27.92 \pm 0.44$ ), overall leaf P concentration (mean of  $0.43 \pm 0.03 \text{ mg g}^{-1}$ ), or any of the other chemical elements analysed (Supplementary Table 3).

#### 3.2 Functional strategy and its influence on leaf stoichiometry after the treatments.

We obtained a species functional strategy gradient by extracting the species' scores from the first and second PCA axes. We then used the two functional strategy gradients in the LMM studying the relationship between changes in leaf element concentration and functional strategy. The first

two phylogenetically corrected PCA axes explained 75 % of the variation in the species' functional traits (Figure 2). The first PCA axis (62 % of the total variance) was characterised by a gradient from acquisitive species with less dense leaves (high SLA) such as *Dracaena* and *Pimelodendron*, to conservative species with dense leaves and dense wood such as *Cotylelobium* and *Tristaniopsis*. The second PCA axis (23 % of the total variance) showed a less clear gradient from species such as *Gaertnera*, with intermediate wood density and growth rate, to species such as *Actinodaphne*, with low wood density and slow growth rate.

Model selection based on AIC revealed that leaf Fe and S concentrations were significantly related to the interaction of treatments with the first PCA axis functional strategy gradient (less to more dense leaves). Leaf Al and Ni concentrations were significantly related to the interaction among treatments and the second PCA axis functional strategy gradient (representing wood density and growth rate) (Table 1; Supplementary Table 3). The change in leaf Fe concentration as a result of CaCO<sub>3</sub> addition was negatively influenced by species SLA, and the change in leaf S concentration was negatively influenced by functional strategy when N was added with, or without, CaCO<sub>3</sub> (Table 1). The changes in leaf Al and Ni concentrations were positively related to functional strategy (as represented by the second PCA axis) after CaCO<sub>3</sub> addition whereas after the combined addition of N and CaCO<sub>3</sub> the change in leaf Al was negatively related to functional strategy as represented by the second PCA axis. There was no significant correlation between the change in leaf N concentration and the interaction of experimental treatments with functional strategy (Supplementary Table 3). For N addition alone, the main effect was significant for three out of five models for N leaf concentration change. These three models, and the equivalent models for C:N ratio changes, were the only models where the main effect of N addition alone was significant, whereas the main effect of CaCO<sub>3</sub> addition alone was not significant in any model (Supplementary Table 3).



We predicted the changes in leaf element concentration for those elements (i.e. Al, Fe, Ni, S) which had a significant interaction with functional strategy, and the relevant experimental treatment. The predicted changes in leaf element concentrations had significant Pearson correlation coefficients with the first (leaf S) and the second (leaf Al) PCA axes and thus corroborated the results from the previous linear mixed model analyses (Figure 3). We consider the results that show a link between the change in leaf elemental composition and the functional PCA axes both in the linear mixed models and in the Pearson correlation as the most conservative, but we cannot ignore that Fe and Ni also had significant links to functional strategy, although this was shown only by the linear mixed model. The elements that had no significant interactions between changes in leaf chemistry and either PCA axis from the linear mixed models or the Pearson correlation analysis are not considered further.

## 4 Discussion

Understanding tropical forest element limitation is fundamental if we are to forecast forests' responses to perturbations in their biogeochemical cycles, which might occur with increasing element addition, e.g. through atmospheric N deposition. In this study, we assessed the response of leaf stoichiometry in a tropical heath forest using a factorial N fertilization and CaCO<sub>3</sub> addition experiment. We determined how species' functional strategy influenced changes in leaf element concentrations and showed that acquisitive and conservative species have different patterns of element uptake after alterations to soil resource availability.

### 4.1 Species element concentration and stoichiometry before the treatments and N-P limitation:

Before the fertilizer application the mean N:P ratio of our leaves from ten species ( $28.7 \pm 0.97$  mg g<sup>-1</sup>) was similar to that found in other Bruneian heath (N:P of 24) and lowland evergreen rain forests (N:P of 31; Metali et al. 2015), but greater than Costa Rican (N:P of 22; Alvarez-Clare and



Mack 2015) lowland evergreen rain forest leaves. Our heath forest leaf N:P ratio, along with the values obtained from other heath forests, are well above the proposed threshold of 16 that Koerselman and Meuleman (1996) suggested would indicate soil P limitation. This contrasts with the general vision of heath forests of being mainly limited by soil N (Proctor et al. 1983; Proctor 1999; Brearley et al. 2011). Dent et al. (2006), using data from a heath forest at a site nearby to ours, proposed that the Kabili-Sepilok heath forest is co-limited by both soil N and P due to a high P-use efficiency. In our study, leaves had a low N concentration (mean  $12.3 \pm 0.89 \text{ mg g}^{-1}$ ) relative to other Bornean lowland rain forests (e.g.  $16.2 \pm 0.71 \text{ mg g}^{-1}$ ; Metali et al. 2015), suggesting our heath site is N deficient. Our species also had a relatively low P concentration (i.e.  $0.44 \pm 0.03 \text{ mg g}^{-1}$ ) compared to  $0.54 \pm 0.04 \text{ mg g}^{-1}$  from Metali et al. (2015). The N:P ratio of 28.7 before the start of our experiment suggests, therefore, that P deficiency is more likely limiting than N deficiency (Townsend et al. 2007; Yan et al. 2017). However, given the relatively low concentration of both N and P in our heath forest leaves, the use of an N:P ratio above 16 to indicate element limitation as proposed by Koerselman and Meuleman (1996) may not be useful. Thus, we believe that the effect of our fertilization experiment on leaf chemistry is a more reliable method to infer plant element limitation than the Koerselman and Meuleman (1996) threshold.

#### **4.2 Response of leaf stoichiometry to the treatments:**

Our experiment highlighted a clear impact of soil fertilization on tree species leaf chemistry after just two years of treatments. In addition to the suggestion above that this forest is limited by P, the overall increase in foliar N, in both conservative and acquisitive species after experimental soil N addition (Figure 1), and the significance of N in the model describing leaf N concentration changes (Supplementary table 3), indicates that trees in this forest are also limited by soil N.

Alternatively, the trees may have absorbed N in excess relative to P, to store it as a form of luxury consumption. Luxury consumption usually occurs in shade-tolerant species (Lawrence 2003), which are ecologically comparable to “conservative” species in our analysis. As we have shown in this heath forest, both acquisitive and conservative species increase their leaf N concentration, and also increased tree growth rate after N addition (Sellan 2019), so we propose that the increase in leaf N concentration in this study suggests ubiquitous N limitation. Following the approach of You et al. (2018) we lean towards an N and P co-limitation hypothesis for heath forest tree growth, as the trees showed an increase in leaf N and an unchanged leaf P concentration after soil N addition, indicative of N and P co-limitation. The N:P ratio, on the other hand, did not increase, possibly because P had a non-significant increase in seven out of ten species. This non-significant trend of co-increasing P with N may be because soil N addition stimulates the activity of phosphatase-producing micro-organisms increasing P availability in soil (Marklein & Houlton 2012). However, as we did not manipulate soil P in our experiment, we are not able to clearly say to what extent this forest is P limited. The lack of a significant overall response of leaf P to fertilization and CaCO<sub>3</sub> addition was an unexpected result. We had expected an increase in leaf P after soil CaCO<sub>3</sub> addition because CaCO<sub>3</sub> reduces soil Al concentration (Badalucco et al. 1992) and the sequestration of soil Al unlocks soil P, making it available to plants (Fageria and Filho 2008). The lack of response in leaf P concentration is possibly due to the low concentration of soil Al in our study site (0.52 cmol<sub>c</sub> kg<sup>-1</sup>; tropical spodosols’ acidity is thought to be dominated by soil H<sup>+</sup> rather than soil Al; Proctor 1999), so soil P is unlikely to be bound with Al. The P limitation inferred from foliar N:P ratios in our heath forest could be due to root stoichiometry. Plant roots absorb P preferentially as orthophosphates (H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and HPO<sub>4</sub><sup>2-</sup>; Raghothama 1999), but this uptake requires the co-transport of H<sup>+</sup>, which may have a toxic effect on roots (Foy 1984). We speculate

that plants might limit the absorption of orthophosphates to avoid a toxic root concentration of  $H^+$ .

#### **4.3 Species functional strategy correlates with response to fertilization:**

Tree species with different functional strategies showed different abilities to acquire soil elements in this studied heath forest. Although current understanding suggests that acquisitive species have a faster rate of uptake of soil elements than conservative species (van der Sande et al. 2018), here, after the  $CaCO_3$  addition, we saw a mixed response. In particular, acquisitive species increased their uptake of soil Al more than conservative species after  $CaCO_3$  addition, and acquisitive species decreased the uptake of soil S compared with conservative species after N addition with and without  $CaCO_3$ . In addition, the LMM suggests an increased Fe uptake by conservative species after  $CaCO_3$  addition. The change in C, Ca, Cu, K, Mg, Mn, Na, P and Zn concentrations did not show any consistently significant interactions with the functional strategy of the trees when considering both the LMM and the Pearson correlation coefficients. Generally, spodosols (such as found in this heath forest) have low cation exchange capacity due to clay eluviation, and also have low concentrations of elements such as Al and Fe (Andriessse 1970; Sobrado 2013), which can be important elements in small quantities. Iron, for example, has been found to influence Bruneian heath forest bacterial communities (Kerfahi et al. 2018) and soil microbial communities in a Belizean calcareous forest (Winbourne et al. 2017). Small concentrations of Al, instead, have increased seedling growth for some Ecuadorian montane forest species (Rehmus et al., 2014). The increased Al concentration in leaves in our study could support the hypothesis of Proctor (1999) who proposed that heath forest productivity was limited by soil acidity (which is driven by  $H^+$  and Al concentration). The  $H^+$  ion is extremely toxic to plants and  $H^+$  toxicity is prominent where soil lacks the buffering activity of Al, as it is the case in extremely weathered spodosols developed on

Al-poor sandstone. In heath forest soils, plant roots limit the absorption of N and P to avoid the co-transport of toxic H<sup>+</sup> ions (Proctor 1999; Luizão et al. 2007; Vernimmen et al. 2013). Thus, an increase in available Al could promote nutrient uptake by replacing H<sup>+</sup> as a co-transported ion. Soil in our experimental forest plots had an extremely low available Fe concentration (mean of 26 µg g<sup>-1</sup>; Sellan et al. 2019) and low exchangeable Al concentration (mean of 0.5 cmol<sub>c</sub> kg<sup>-1</sup>; Sellan et al. 2019). This is much lower than in the CaCO<sub>3</sub> we used for our experiment that we found had high Al (6420 µg Al g<sup>-1</sup>; Sellan 2019) and Fe concentrations (11,530 µg Fe g<sup>-1</sup>; Sellan 2019). The increased uptake of Fe by conservative species might indicate that when these plants grow in an element poor soil such as our heath forest spodosol, they utilize a form of biochemical stress tolerance. Heath forests are often more severely affected by adverse environmental stresses such as drought and high irradiance (Bowler et al. 1994) than mixed forest (Miyamoto et al. 2007). Iron is fundamental for reduction in environmental stress through free radical detoxification associated with environmental stress (e.g. through the enzyme superoxide dismutase. Uchida 2000). Species with conservative traits are generally longer lived and more stress tolerant than acquisitive species (Poorter and Bongers 2006; Ruger et al. 2012; Lohbeck et al. 2013), particularly to drought (van der Sande et al. 2018), and shade (Baltzer and Thomas 2010). Few results on the effects of CaCO<sub>3</sub> addition on plant Fe concentrations are available from the literature, but in North American acid soils (pH<sub>CaCl2</sub> = 4.8), CaCO<sub>3</sub> addition has been shown not to affect, or even reduce the foliar Fe concentration of *Festuca arundinacea* (Hamilton 2006), possibly due to soil Fe sequestration by carbonates (Brown 1961). The decrease in leaf S concentration after N addition seems counterintuitive. Sulphur is a constituent element for several amino acids and has a stoichiometric ratio with N of 1:15 in cultivated plants (Jamal et al. 2010), thus an increase in leaf N should also promote an increase in leaf S. As an additional result, we found that *G. junghuhniana* is an Al accumulator, as it has high concentration of Al (highest value of 5.62 mg g<sup>-1</sup>, mean value of 2.72 ±

1.65 mg g<sup>-1</sup> compared to 0.08 ± 0.02 mg g<sup>-1</sup> for all the other species). The ability of *G. junghuhniana* to accumulate Al could explain its widespread distribution in our Al poor soil.

Given the increasing input of N into tropical forest in this region we were interested in finding out if this Bornean heath forest was N limited and what the potential impact might be on ecosystem functioning of increased N on these forests. Our experiment has shown that all species in this heath forest increased their N uptake after N fertilization. We also show that species at the two ends of the acquisitive to conservative plant functional strategy gradient have different Al, Fe Ni and S uptake. The change in the forest's soil pH after N deposition alters element availability, so, depending on the element, increasing N pollution and atmospheric deposition, could lead to losses of functional diversity and biodiversity in forests, including this rare heath forest, and across landscapes. We stress here the importance of conducting further studies to investigate the impact of N deposition and resultant biochemical changes to soil in tropical forests.

**Acknowledgements:** This research was funded by Manchester Metropolitan University's Environmental Science Research Centre. We would like to thank the Sabah Biodiversity Council, the Soil Chemistry section, Ecology section and Herbarium staff from Sabah Forest Research Centre and in particular the research assistants Juanis Runcin, Jemson Jumian, Postar Miun, Spincer Sitim, Lioba Sawadon, Yun Len Lee and the head of Soil Chemistry section Rolando Robert. We are indebted to David McKendry and Graham Tinsley for the assistance in the Manchester Metropolitan University laboratory, to Nancy Dise, Chris Field, Liam Trethowan and Matteo Giacomazzo for valuable suggestions and Daniela Krebber for the assistance with coding.

**Authors Contribution:**

F.Q.B., G.S. and J.T. designed the experiment, G.S. conducted the fieldwork, lab work and analysed the data, N.M. provided logistical support and intellectual input, G.S. and F.Q.B. wrote the first draft of the paper and all authors revised the manuscript.

**Conflicts of interest:**

The authors declare they have no conflict of interests.

**Data accessibility:**

The data are freely available on the Natural Environment Research Council (NERC) Environmental Information Data Centre (<https://doi.org/10.5285/d5ea9e2c-f053-4631-aead-a8a5557488c6>).

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## 6 Tables and figures

Table 1. Results of the selected linear mixed models studying the relation between the changes in leaf elemental concentrations after two years of experimental addition (N, CaCO<sub>3</sub> or N+CaCO<sub>3</sub>) and functional traits (PC1 and PC2) in a Malaysian heath forest. Significant interactions are in bold and significance symbols are as follows: '\*\*\*\*' <0.001, '\*\*\*' <0.01, '\*\*' <0.05. The full table is available in Supplementary information (Supplementary Table 3).

Element	Structure	Estimate	Std. Error	t value
Al	Intercept	0.33	0.07	4.89**
	N	0.12	0.10	1.25
	CaCO <sub>3</sub>	-0.03	0.10	-0.30
	<b>PC2</b>	<b>0.03</b>	<0.01	<b>8.45**</b>
	N*CaCO <sub>3</sub>	-0.20	0.15	-1.34
	N*PC2	0.01	0.01	1.21
	<b>CaCO<sub>3</sub>*PC2</b>	<b>0.01</b>	<b>0.01</b>	<b>2.20*</b>
	<b>N*CaCO<sub>3</sub>*PC2</b>	<b>-0.02</b>	<b>0.01</b>	<b>-2.33*</b>
Fe	Intercept	3.10E-02	5.30E-03	5.89**
	N	3.10E-03	2.90E-03	1.07
	CaCO <sub>3</sub>	4.40E-03	3.00E-03	1.46
	<b>PC1</b>	<b>3.00E-04</b>	<b>4.90E-05</b>	<b>6.04**</b>
	N*CaCO <sub>3</sub>	-5.10E-03	4.20E-03	-1.20
	N*PC1	1.70E-05	7.60E-05	0.22
	<b>CaCO<sub>3</sub>*PC1</b>	<b>-2.20E-04</b>	<b>7.70E-05</b>	<b>-2.90**</b>
	N*CaCO <sub>3</sub> *PC1	-1.10E-06	1.10E-04	-0.01
Ni	Intercept	2.00E-03	6.00E-04	3.36*
	N	-2.00E-04	6.00E-04	-0.28
	CaCO <sub>3</sub>	4.00E-04	6.00E-04	0.75
	PC2	-3.00E-06	2.00E-05	-0.15
	N*CaCO <sub>3</sub>	-3.00E-04	8.00E-04	-0.39
	N*PC2	-2.00E-05	3.00E-05	-0.62
	<b>CaCO<sub>3</sub>*PC2</b>	<b>1.00E-04</b>	<b>3.00E-05</b>	<b>2.87**</b>
	N*CaCO <sub>3</sub> *PC2	-9.00E-05	5.00E-05	-1.87
S	Intercept	1.62	0.08	19.09**
	N	-0.20	0.10	-2.02
	CaCO <sub>3</sub>	-0.08	0.10	-0.78
	<b>PC1</b>	<b>0.03</b>	<0.01	<b>13.18**</b>
	N*CaCO <sub>3</sub>	0.16	0.15	1.08
	<b>N*PC1</b>	<b>-0.01</b>	<0.01	<b>-3.04**</b>
	CaCO <sub>3</sub> *PC1	<0.01	<0.01	-1.17
	<b>N*CaCO<sub>3</sub>*PC1</b>	<b>0.01</b>	<0.01	<b>2.23*</b>

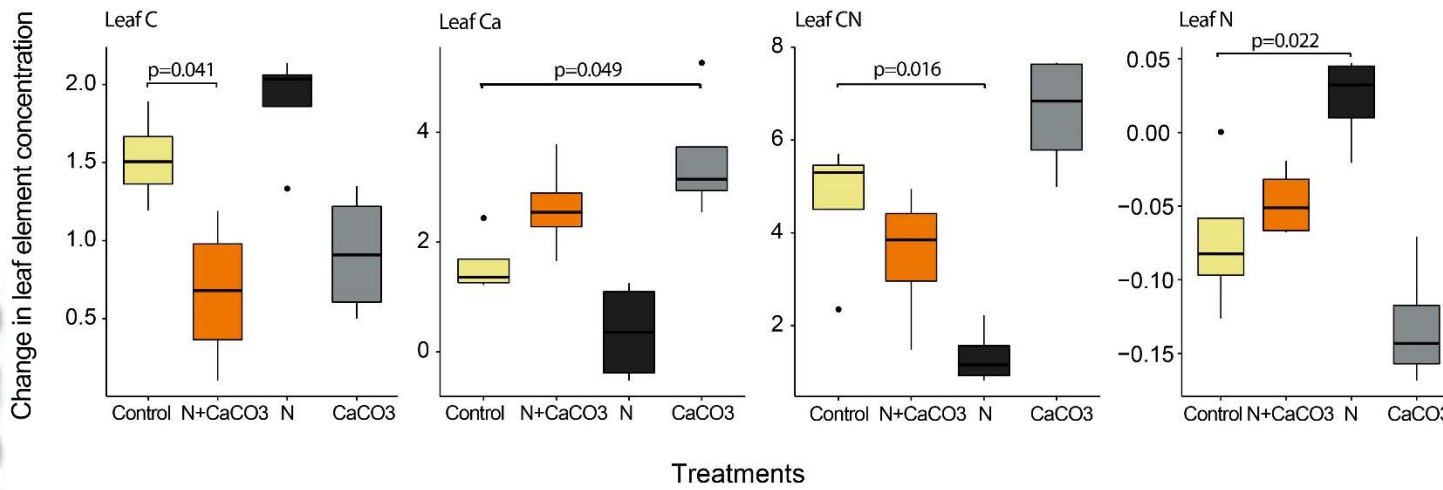


Figure 1. Change in selected leaf element concentrations (plot means combined for ten common tree species) after two years of experimental nutrient addition in a Malaysian heath forest. We calculated significance with ANOVA and Tukey HSD test. Leaf C, CN and N are expressed in % and Ca is expressed in  $\text{mg g}^{-1}$

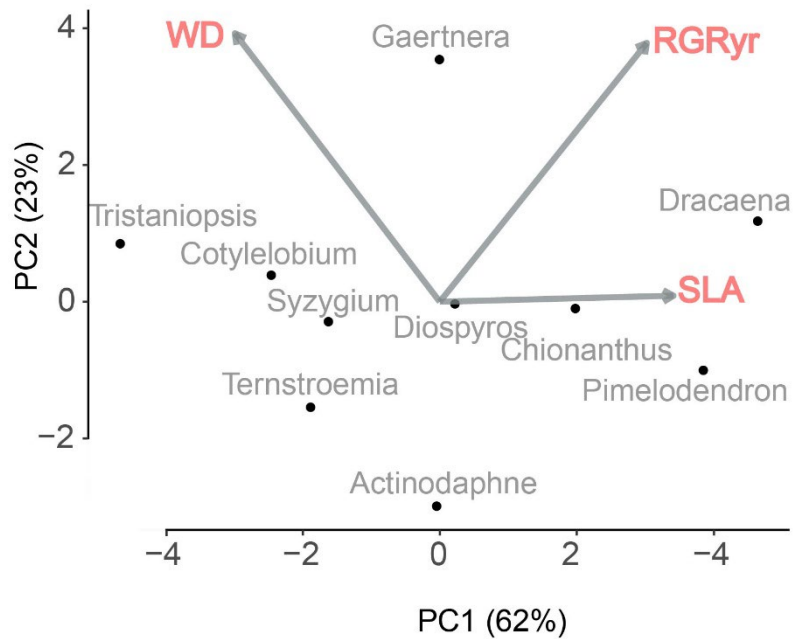


Figure 2. Phylogenetically controlled principal component analysis of three functional traits among ten target species from a Malaysian heath forest used for an experimental element addition study. SLA = specific leaf area, RGRyr = annual relative stem diameter growth rate, WD = wood density.

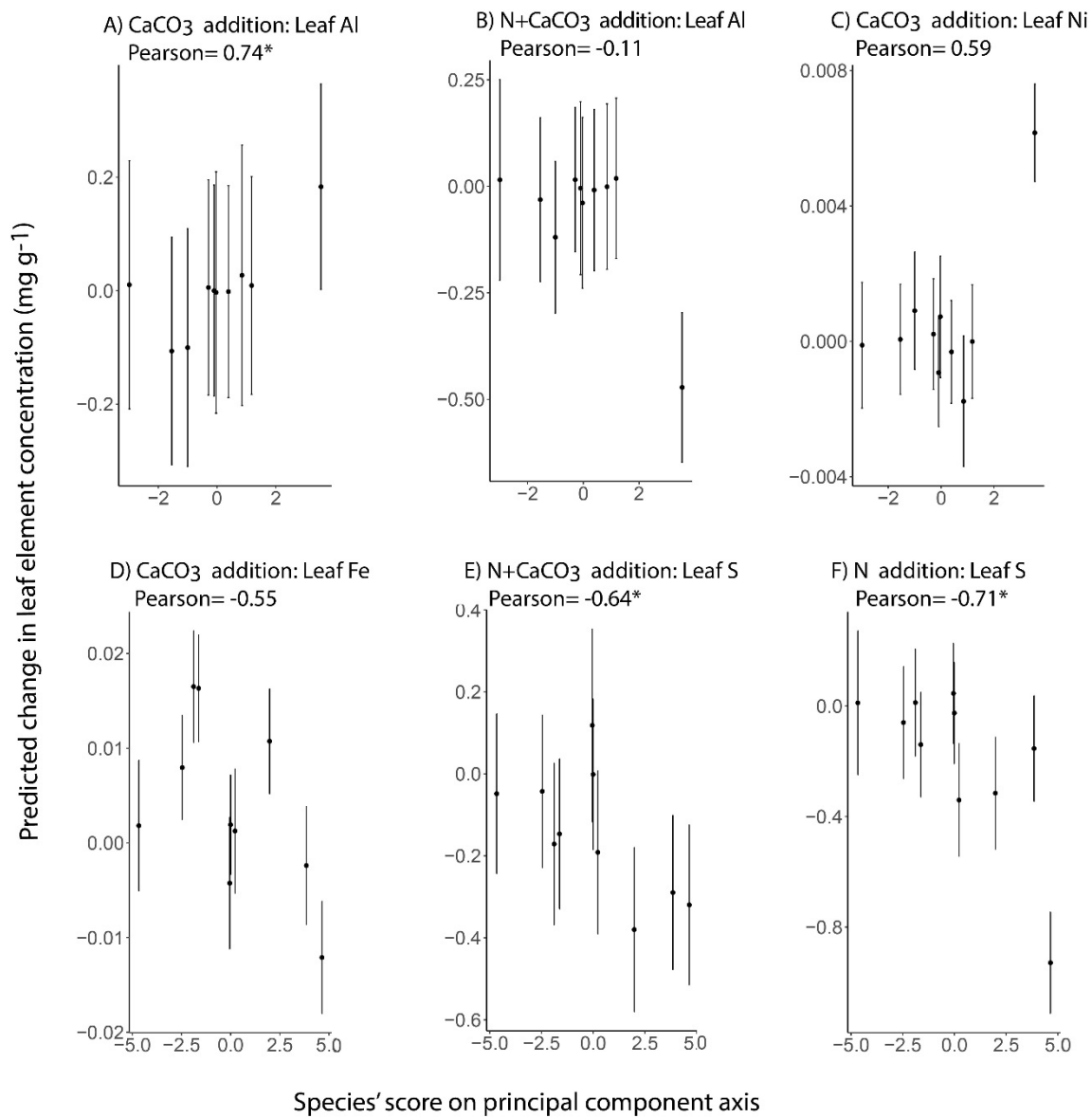


Figure 3. Relationship between the predicted change in leaf elemental concentrations with functional strategy for ten target species from a Malaysian heath forest. The mean values and standard deviations of the changes in element concentration are predicted by the linear mixed models. In figure A), B) and C) species are ordered following their score on the second principal component axis: *Actinodaphne borneensis*, *Ternstroemia aneura*, *Pimelodendron griffithianum*, *Syzygium sp.*, *Chionanthus pluriflorus*, *Diospyros fusiformis*,



*Cotylelobium melanoxydon*, *Tristaniopsis obovata*, *Dracaena elliptica*, *Gaertnera junghuhniana*. In figure D), E) and F) species are ordered following their score on the first principal component axis: *Tristaniopsis obovata*, *Cotylelobium melanoxydon*, *Ternstroemia aneura*, *Syzygium sp.*, *Actinodaphne borneensis*, *Gaertnera junghuhniana*, *Diospyros fusiformis*, *Chionanthus pluriflorus*, *Pimelodendron griffithianum*, *Dracaena elliptica*. The correlation and its significance are computed with Pearson test.