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1 **Nitrogen and phosphorus availability interact to modulate leaf trait scaling**  
2 **relationships across six plant functional types in a controlled-environment study**

3

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18

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32

33

34 **Summary**

35 • Nitrogen (N) and phosphorus (P) have key roles in ~~plant-leaf~~ metabolism resulting in a strong  
36 coupling of ~~leaf~~ chemical composition traits to metabolic rates in field-based studies.

37 However, in such studies, it is difficult to disentangle the effects of nutrient supply *per se* on  
38 trait-trait ~~scaling~~ relationships.

39 • Our study assessed how high and low N (5 mM and 0.4 mM respectively) and P (1 mM and 2  
40  $\mu$ M P respectively) supply on 37 species from six plant functional types (~~PFT~~) affected  
41 photosynthesis (A) and respiration (R) (~~[in darkness ( $R_{Dark}$ ) and light ( $R_{Light}$ )]~~) in a controlled-  
42 environment.

43 • Low P supply only reduced increased scaling-exponents (slopes) of area-based the log-log A-N  
44 or R-N relationships when N supply was not limiting (high N), whereas there was no P-effect  
45 in under low N supply-grown plants. By contrast, scaling-exponents ~~slopes~~ of A-P and R-P  
46 relationships were altered by ~~both~~ P and N supply, ~~with lower N resulting in lower metabolic~~  
47 ~~rates for a given P. While N~~ Neither the R:A ratio nor light inhibition of leaf R the  $R_{Light}:R_{Dark}$   
48 ratio was affected by nutrient supply. Light inhibition ~~of respiration~~ was 26% across  
49 nutrient treatments; but he herbaceous species ~~had~~ exhibited, though some PFT differences  
50 occurred, a lower degree of light inhibition than woody species. ~~Slopes of A-N and R-N~~  
51 ~~relationships were lower in plants grown on low P and high N, whereas there was no P-effect~~  
52 ~~in low N-grown plants. As a function of leaf P, slopes of bivariate relationships were altered by~~  
53 ~~both P and N supply, with lower N resulting in lower metabolic rates for a given P. Neither the~~  
54 ~~R:A ratio nor the  $R_{Light}:R_{Dark}$  ratio was affected by nutrient supply, though some PFT~~  
55 ~~differences occurred.~~

56 • Because ~~variations in~~ N and P supply modulates leaf trait-trait relationships, it is vital to  
57 characterise the mechanistic impact of nutrient supply *per se* on these relationships given  
58 their application in the next generation of terrestrial biosphere models may need to consider  
59 how limitations in N and P availability affect trait-trait relationships when predicting carbon  
60 exchange using leaf chemical composition need to incorporate nutrient variations across  
61 biomes.

62

63

64 **Keywords:** day respiration, glasshouse, leaf economic spectrum, nitrogen, nutrient supply,

65 ~~phosphorus~~, photosynthesis, plant functional groups.

## 66 Introduction

67 Nitrogen and phosphorus are both essential plant nutrients involved in many important plant  
68 metabolic functions, including rates of light-saturated photosynthesis ( $A$ ) and leaf respiration ( $R$ ).  
69 Strong positive relationships are found between both  $A$ ,  $R$  and foliar N (Field & Mooney, 1986;  
70 Evans, 1989; Ryan, 1995; Reich *et al.*, 2008), reflecting the presence of N in key proteins. As a result,  
71 N deficiencies can ~~have negative effects on~~ reduce capacity of photosynthetic and respiratory  
72 enzymes, leading to lower leaf  $\text{CO}_2$  exchange (Evans & Terashima, 1988; Noguchi & Terashima,  
73 2006). Phosphorus (P) is a major component of nucleic acids, ATP, sugar phosphates and  
74 phospholipids, all of which are intrinsically related to photosynthesis and respiration (Rao & Terry,  
75 1995). Consequently, P-deficient plants often exhibit: reduced stomatal conductance (Thomas *et*  
76 *al.*, 2006); reduced ribulose biphosphate (RuBP) regeneration, carboxylation activity and  $A$   
77 (Bloomfield *et al.*, 2014; Ellsworth *et al.*, 2015); and, lower allocation of N to RuBP  
78 carboxylase/oxygenase (Rubisco) (Warren & Adams, 2002). Increased N allocation to non-  
79 photosynthetic components and/or inactivation of Rubisco are additional factors that might  
80 contribute to lower  $A$  under P-deficiency (Stitt & Schulze, 1994). Phosphorus also plays a crucial role  
81 in regulating glycolysis and mitochondrial electron transport (Plaxton & Podesta, 2006), with low P  
82 supply having the potential to result in reduced  $R$  (Gonzalez-Meler *et al.*, 2001). Limitations in P  
83 supply can lead to accumulation of starch (Hammond & White, 2008), ~~and~~ increases in dry mass  
84 per unit leaf area ( $M_a$ ) (Fyllas *et al.*, 2009; Turnbull *et al.*, 2016). Importantly, the effect of P-  
85 deficiency on ~~such plant~~ traits is likely to differ depending on whether P is more or less limiting than  
86 N, ~~reflecting Liebig's law of the minimum~~ (e.g. Aerts *et al.*, 1992; Meir *et al.*, 2001) (Ågren *et al.*,  
87 2012).

88 The coupling of metabolism to leaf chemical composition is important for predictions of  
89 plant carbon exchange at a global scale ~~(Sitch *et al.*, 2008)~~ (Kattge *et al.*, 2009), with '~~scaling~~  
90 ~~relationships' having been developed linking~~ variation in metabolic rates being linked to variation  
91 in  $M_a$  and mass-based leaf N ( $N_m$ ) (Wright *et al.*, 2004; Kattge *et al.*, 2011). Plants growing on  
92 nutrient-poor soils often have longer lived leaves, higher  $M_a$  values, lower  $N_m$ , and lower mass-  
93 based  $A$  ( $A_m$ ) and leaf  $R$  in darkness ( $R_{\text{Dark},m}$ ) than plants growing on nutrient-rich soils ~~(Wright &~~  
94 ~~Westoby, 2003)~~ (Wright *et al.*, 2003; Wright & Westoby, 2003). When trait-trait relationships are  
95 compared for sites differing in nutrient availability, distinct patterns are often observed. For  
96 example, the slope of bivariate-linear relationships-regressions linking  $A$  to leaf  $N_m$  are often lower

97 in plants growing on low P-soils (Reich & Walters, 1994; Denton *et al.*, 2007; Kattge *et al.*, 2009).  
98 Comparing tropical leaf traits at sites differing in P availability, Kattge *et al.* (2009) reported lower  
99 rates of area-based maximum carboxylation rates ( $V_{\text{cm},a}$ ) at given area-based N ( $N_a$ ) in plants  
100 growing on low P soils. Similarly, the slope (or scaling-exponent) of the log-log A-N relationships is  
101 lower in tropical trees growing on low P soils than temperate trees growing on high P soils (Reich *et al.*, 2009).  
102 Moreover, Bahar *et al.* (2016) reported lower N-based rates of A and  $V_{\text{cm},a}$  in tropical  
103 rainforest species growing on low P soils in Peru (compared to high P soils). Collectively, these field-  
104 based studies suggest that coupling of photosynthetic metabolism with N may depend on P-  
105 availability and hence a single 'universal' scaling relationships may not be realistic.

107 Relationships between leaf N and dark respiration are also affected by P-supply. Meir *et al.*  
108 (2001) found that area-based leaf  $R_{\text{Dark}}$  ( $R_{\text{Dark},a}$ ) at a given  $N_a$  being higher at P-limited sites. By  
109 contrast, Rowland *et al.* (2016) reported higher area-based  $R_{\text{Dark}}$  ( $R_{\text{Dark},a}$ ) at a given  $N_a$  in tropical  
110 trees growing on low nutrient soils; this study also found that  $R_{\text{Dark},a}$  was less affected by nutrient  
111 limitations than A, resulting in higher R:A ratios in plants growing on nutrient-deficient soils.  
112 Further, Reich *et al.* (2006) found that the log-log relationship between whole-plant  $R_{\text{Dark},m}$  and  $N_m$   
113 was unaffected by nutrient supply, a finding also observed at the leaf-level (Atkin *et al.*, 2013). In  
114 comparisons of log-log plots, Wright *et al.* (2004) showed intercept-elevation (proportionality-  
115 coefficient) differences for among sites differing in nutrient availability but with constant  $R_{\text{Dark},m}$ - $N_m$   
116 slopes-scaling-exponents across sites. Notwithstanding the contrasting observations of these  
117 studies, what is clear is that there is no single  $R_{\text{Dark}}$ -leaf N relationship. What is less clear, however,  
118 is what role soil N and P play in determining rates of metabolism and relationships linking A and R  
119 to other leaf traits.

120 ~~H~~As noted above, some studies assessing impact of nutrient availability on trait-trait  
121 relationships have used linear relationships fitted to untransformed data, while others have log  
122 transformed data on both axes. (e.g. Atkin *et al.*, 2013) How might deficiencies in N and/or P  
123 affect log-log relationships fitted to multi-species data sets made up of contrasting across? When  
124 grown on limiting N supply, leaf N concentration can decrease to a greater extent than does the  
125 rate of metabolism, underpinned by an increase in the proportion of N allocated to metabolic  
126 processes (Makino *et al.*, 1994; Pons *et al.*, 1994; Cheng & Fuchigami, 2000); in such cases, low N  
127 treatment would result in higher rates of A per unit leaf N (i.e. higher  $PNUE_{A_N}$ ). If the proportional

128 increase in  $A_N$  is similar among species, then only the proportionality-coefficient of log-log A-N  
129 plots would change (see Supporting Information Notes S1 for further details). However, if  
130 proportional changes in  $A_N$  are greater in some species than others, ~~although may  $A_N$~~  (e.g. Warren  
131 *et al.*, 2003; Pons & Westbeek, 2004; Hikosaka, 2010), then limitations in N supply might alter the  
132 scaling-exponent of log-log A-N relationships. (~~Warren *et al.* 2003, (Pons & Westbeek, 2004;~~  
133 ~~Hikosaka, 2010) For example, Warren *et al.* (2003) found that the maximal rate of carboxylation by~~  
134 ~~Rubisco ( $V_{\text{cmax}}$ ) per unit leaf N of *Pinus sylvestris* remained constant irrespective of N supply; others~~  
135 ~~have reported similar observations (Pons & Westbeek, 2004; Hikosaka, 2010). Thus, it is uncertain~~  
136 ~~how limitations in N supply affect the slope and/or elevation of log-log trait-trait relationships for~~  
137 ~~data sets that include a wide range of species differing in leaf structure/chemistry and life-history~~  
138 ~~characteristics~~ Similar issues are at play for how low P supply impacts on log-log A-N relationships,  
139 depending on: (1) the extent to which leaf phosphorus can be re-allocated from non-metabolic to  
140 metabolic pools (Woodrow *et al.*, 1984; Foyer & Spencer, 1986; Raghothama, 1999); (2) whether  
141 low P supply impacts on N allocation within leaves (Whitehead *et al.*, 2005; Reich *et al.*, 2009); and  
142 (3) the extent to which these responses differ among species ~~representative of~~ on the leaf economic  
143 spectrum (Wright *et al.*, 2004).

144 Leaf respiration takes place both in the light ( $R_{\text{Light}}$ ) and the dark, with  $R_{\text{Light}}$  typically lower  
145 than  $R_{\text{Dark}}$  (Way *et al.*, 2015), even when accounting for re-fixation of respiratory  $\text{CO}_2$  (Pärnik *et al.*,  
146 2007). Failure to account for light-induced inhibition of leaf  $R$  can lead to overestimation of  
147 ecosystem respiration (Wehr *et al.*, 2016). The extent of inhibition is highly variable, ranging from  
148 80% inhibition through to cases where  $R_{\text{Light}}$  is 30% higher than  $R_{\text{Dark}}$  (Heskel *et al.*, 2014; Way &  
149 Yamori, 2014). While the mechanisms underpinning light inhibition are not fully understood,  
150 photorespiratory metabolism is likely involved (Randall *et al.*, 1990; Tcherkez *et al.*, 2008).

151 Although the impact of nutrient gradients on light inhibition has been examined in a few field  
152 studies (Atkin *et al.*, 2013; Heskel *et al.*, 2013), little if any attention has been given to the main and  
153 interactive effects of N and P deficiency on the degree of light inhibition, or how N and P supply  
154 affect scaling relationships linking leaf  $R_{\text{Light}}$  to related traits. In addition, no study has yet addressed  
155 the question of whether there are systematic differences in light inhibition among species or plant  
156 functional types (PFTs). PFTs are used in terrestrial biosphere models (TBMs) as the starting point  
157 for predicting metabolic rates across the globe, with TBM-dependent foliar N often being the  
158 starting point for predicting rates of  $A$  and  $R$  (Clark *et al.*, 2011; Oleson *et al.*, 2013).

159 Given the importance of leaf trait relationships for the functioning of individual plants and  
160 whole ecosystems, with strong tendencies for leaf N and P to co-vary in nature (Sterner & Elser,  
161 2002; Reich & Oleksyn, 2004), it is difficult to isolate the effects of low P from low N in explaining  
162 broad patterns of variation in relationships between metabolism and leaf nutrients. This is  
163 especially so in field studies comparing species from among biomes, and among sites within  
164 individual biomes (Kattge *et al.*, 2009; Reich *et al.*, 2009; Domingues *et al.*, 2015). Given these  
165 challenges, we used a factorial design using 37 species from six PFTs to isolate the individual and  
166 combined effects of N and P availability supply on a range of leaf traits associated with  
167 photosynthesis and respiration. Our study provides the first multi-PFT comparison assessing  
168 nutrient impacts on leaf trait relationships under controlled environment conditions. ~~and the fact~~  
169 ~~that nutrient availability varies markedly in nature, it is vital that the impacts of nutrient supply per~~  
170 ~~se on leaf trait relationships be characterised. Past studies assessing impacts of N and P~~  
171 ~~availability on trait relationships have been field-based and, as such, not able to definitively~~  
172 ~~assess the effects of nutrient supply on leaves. It was with this in mind that we conducted a~~  
173 ~~controlled environment study using 37 species from six PFTs, with each species grown on high and~~  
174 ~~low N and P supply.~~ We tested the following hypotheses:

- 175 1. -Growth under low P supply will reduce average rates of leaf A and R, with the inhibitory  
176 effect being greater in high N ~~than low N~~-grown plants, ~~but have limited minimal effect in~~  
177 ~~plants grown on when N is limiting limiting N, reflecting Liebig's law of the minimum.~~  
178 Similarly, the deleterious effect of low N supply on A and R will be less pronounced when P  
179 is limiting. Such effects are expected to be similar among PFTs. ~~Similarly, the deleterious~~  
180 ~~effect of low N supply on A and R will be less pronounced when P is limiting.~~
- 181 2. Irrespective of P supply, limitations in N supply will the elevation (i.e. y-axis intercept) of log-  
182 ~~log A-N,  $R_{\text{Dark-N}}$  and  $R_{\text{Light-N}}$  relationships will be higher when plants are grown on limiting N~~  
183 ~~supply (Fig. 1).~~ Wincrease the proportionality-coefficient of log-log A-N,  $R_{\text{Dark-N}}$  and  $R_{\text{Light-N}}$   
184 relationships, but not alter the scaling-exponent (Fig. 1). ~~-limiting conditions~~
- 185 3. ~~Irrespective of N supply,~~ P deficiency will the slope of ~~reduce the proportionality-coefficient~~  
186 ~~of log-log A-N,  $R_{\text{Dark-N}}$  and  $R_{\text{Light-N}}$  relationships, with the proportional decline in metabolic~~  
187 ~~rates at any given N being greater on high N supply than under low N supply~~ -will be lower  
188 ~~when plants are grown on limiting P supply (Fig. 1).~~



- 189 4. R:A ratios will be greater for nutrient-limited plants ~~for~~ compared to plants grown on high N  
190 and P supply, reflecting the greater inhibitory effect of limiting nutrient supply on  
191 photosynthesis compared to that of respiration (Evans & Terashima, 1988).
- 192 5. ~~(Shapiro et al., 2004; Tcherkez et al., 2008; Ayub et al., 2011; Crous et al., 2012; Griffin &~~  
193 ~~Turnbull, 2013)~~ Nutrient treatments that reduce rates of photorespiration (i.e. limitations in  
194 N and/or P supply) will ~~may~~ will result in lower ~~There is no systematic difference in the~~  
195 degrees of light inhibition of leaf ~~R~~ ~~among species or nutrient treatments~~ (see SI Notes S2  
196 for further details).

## 199 **Materials and Methods**

### 201 *Plant material and experimental design*

202 The study comprised 20 woody and 17 herbaceous species from 17 families, representing six PFTs:  
203 broadleaved trees (~~B~~BTL), broadleaved shrubs (S), coniferous needle-leaved trees (~~N~~NLT), C<sub>3</sub>  
204 grasses (C3G), C<sub>3</sub> herbs (C3H) and C<sub>4</sub> grasses (C4G) (Table S1). Four Protaceae species which can  
205 access P more efficiently under low P (Lambers *et al.*, 2010) were included in the woody species  
206 group (Table S1). The woody species were planted in November 2008, and measured starting in  
207 January 2009, whereas the herbaceous species were planted in November 2009 and measured in  
208 January 2010 onwards. Woody plants were sourced mainly from the Wagga Wagga Forestry  
209 Nursery (Wagga Wagga, NSW, Australia) whereas the herbaceous species were sourced from the  
210 Woodbridge nursery (Woodbridge, Tasmania) or grown from seeds (Nindethana Australian Seeds,  
211 Albany, WA, Australia). Twenty-four plants of each species were potted in sterilised sand in 50 cm  
212 tall PVC, 9 cm diameter, 3.18 L pipes to achieve six replicates per nutrient treatment. Two adjacent  
213 glasshouses were used with three replicate blocks in each glasshouse. Species and nutrient  
214 treatments were randomly allocated within each block. Species were grown in the glasshouse  
215 under natural light conditions at 25°C/18°C (day and night).

216 Nutrient treatments represented four combinations of different N and P concentrations: High  
217 N–High P (H<sub>N</sub>H<sub>P</sub>), High N–Low P (H<sub>N</sub>L<sub>P</sub>), Low N–High P (L<sub>N</sub>H<sub>P</sub>) and Low N–Low P (L<sub>N</sub>L<sub>P</sub>). The H<sub>N</sub>H<sub>P</sub>  
218 solution was based on Hoagland No. 1 solution (Hoagland & Arnon, 1950) with the following  
219 macronutrient composition: 1 mM KH<sub>2</sub>PO<sub>4</sub>, 5 mM KNO<sub>3</sub>, 0.07 mM CaCl<sub>2</sub>, 0.45 mM MgSO<sub>4</sub>·7H<sub>2</sub>O. The

220 ~~Whereas the final concentrations of 'high N' solutions were 5 mM KNO<sub>3</sub>,~~ 'low N' solutions had a  
221 ~~final~~ concentration of 0.4 mM KNO<sub>3</sub> (modified after Atkinson *et al.*, 2007). The 'high P' solutions  
222 contained 1 mM KH<sub>2</sub>PO<sub>4</sub> (Edwards *et al.*, 2006) whereas 'low P' had 2.0 μM KH<sub>2</sub>PO<sub>4</sub> to limit storage  
223 of P in the vacuole as a buffer (after Campbell & Sage, 2006). Thus, N:P supply ratios varied from 5:1  
224 for H<sub>N</sub>H<sub>P</sub> to 2500:1 for H<sub>N</sub>L<sub>P</sub>, 0.4:1 for L<sub>N</sub>H<sub>P</sub> and finally 200:1 for L<sub>N</sub>L<sub>P</sub>. Here, our aim was to achieve  
225 changes in foliar chemistry and function, that was also reflected in rates of leaf ~~plant~~ metabolism,  
226 rather than mimic naturally occurring N:P ratios ~~as such rather than mimic natural soil N:P ratios.~~

227 Each nutrient solution was balanced for cations and contained the same amount of micronutrients  
228 and FeEDTA. Micronutrients were constant in all solutions: 4.2 μM B, 1.2 μM Mn, 0.8 μM Zn, 0.03  
229 μM Cu, 0.04 μM Mo and 0.01 μM Co. Iron was added as ferric EDTA to a level of about 8 μM Fe.  
230 These micronutrient concentrations were one-tenth of the recommended Hoagland solution  
231 because full strength has resulted in toxic symptoms (Leggett, 1971). Nutrient solutions were made  
232 up in 200 L black storage tubs, which were refilled regularly. Once per day, nutrient solutions were  
233 pumped out of 200 L tubs and delivered to the pots using irrigation tubing and drippers at 20 ml per  
234 minute. Plants received on average about 120-150 mL solution each day and were watered at the  
235 end of each day.

### 237 *Gas exchange measurements*

238 Gas exchange was measured between 9 am and 2:30 pm with portable infrared gas analysers (LiCor  
239 6400, LiCor Inc., NE, USA). 873 light-response curves were conducted ~~on~~ on the most recently fully  
240 developed leaves in each treatment ~~at a single timepoint~~, starting with light-saturating conditions  
241 (irradiance: 1800 μmol m<sup>-2</sup> s<sup>-1</sup>; flow: 500 μmol s<sup>-1</sup>; leaf temperature: 25°C; reference CO<sub>2</sub>: 400 μmol  
242 mol<sup>-1</sup>). After these initial conditions, light was decreased to 1500 μmol m<sup>-2</sup> s<sup>-1</sup> and then 100 μmol m<sup>-2</sup>  
243 s<sup>-1</sup>, and finally in steps of 5-10 μmol m<sup>-2</sup> s<sup>-1</sup> to achieve estimates of respiration during the day  
244 ( $R_{\text{Light}}$ ) using the Kok method (Kok, 1948).  $R_{\text{Dark}}$  was obtained 10 minutes after the light was turned  
245 off (flow 300 μmol s<sup>-1</sup>) to avoid post-illumination transients (Atkin *et al.*, 1998). For each leaf,  
246 measurements were made at a single time point.

247 The Kok effect refers to the break in the slope of plots of net CO<sub>2</sub> uptake vs irradiance near  
248 the light-compensation point (Ayub *et al.*, 2011). Using this approach, a regression line is extrapolated  
249 from the linear part of the light-response curve (over the irradiance range 20-60 μmol m<sup>-2</sup> s<sup>-1</sup>), with  
250 the y-axis intercept representing  $R_{\text{Light}}$ .  $R_{\text{Light}}$  was corrected for changes in  $C_i$  (intercellular CO<sub>2</sub>

251 concentration) associated with changes in irradiance (Kirschbaum & Farquhar, 1987). All curves were  
252 analysed using R (R Core Development Team, 2015).

253

#### 254 *Structural and chemical leaf composition*

255 Leaves were collected after each light response curve and the leaf area inside the leaf cuvette was  
256 measured before drying at 70°C for at least 24 h to determine leaf mass per area ( $M_a$ ). Total N and  
257 P were determined via Kjeldahl digestion (Allen, 1974), with digests analysed using a flow injection  
258 analyser (Lachat instruments, Loveland, Colorado, USA) for N and P using the indophenol blue  
259 (Scheiner, 1976) and ammonium molybdate methods (John, 1970) respectively. Leaves from 16  
260 woody species were analysed for soluble sugars and starch according to Loveys *et al.* (2003).

261

#### 262 *Statistical Analyses*

263 ~~Initial data exploration revealed cases of outlying (but not erroneous) values, unequal variation~~  
264 ~~among treatment classes and non-normal distributions. Many of our variables, notably leaf N and~~  
265 ~~P, showed distributions that were heavily right-skewed and therefore. For consistency of~~  
266 ~~presentation and interpretation all variables have been log-transformed in the analyses that follow.~~  
267 ~~Log transformation was the best option chosen from a number of alternative methods (e.g. square~~  
268 ~~root and inverse) and that with improvement applied both to the underlying variables and to model~~  
269 ~~residuals].~~ Because most variables followed a rightly skewed distribution, values were log-  
270 transformed before screening for outliers and subsequent analysis, as outlined in SI SM1. ~~Data were~~  
271 ~~filtered for outliers identified as those falling beyond twice the interquartile range with for a given~~  
272 ~~variable. Filters for separate variables were applied to the dataset in the following order:  $P_m$ ,  $N_m$ ,~~  
273  ~~$M_a$ ,  $R_{Dark,m}$  and  $A_m$ . Application of these filters resulted in a total of 15 outliers/rows being removed~~  
274 ~~from the dataset. Whenever an outlier was identified, the entire observational row was removed.~~  
275 ~~Application of these filters resulted in a total of 15 outliers being removed from the dataset. After~~  
276 ~~filtering, 37 species, six PFTs, and four nutrient treatments remained (Table S1) totalling 873~~  
277 ~~measurements (with 148 PFT species by treatment combinations). Woody species represented 472~~  
278 ~~observations. Further filtering was necessary for  $R_{Light}$ , removing three species (*Banksia spinulosa*,~~  
279 ~~*Pinus halepensis* and *Banksia integrifolia*) due to inadequate replication or measurements with~~  
280 ~~negative  $R_{Light}$  values (i.e. positive y-axis intercepts elevation from Kok estimates — see~~  
281 ~~above method). Data falling beyond twice the interquartile range resulted in further removal of~~

282 ~~twelve rows. Hence the  $R_{\text{Light}}$  dataset consisted of 710 values.~~ Calculations of means and ANOVA  
283 tests were conducted in JMP (SAS Institute, NC). We used individual plant data when comparing  
284 bivariate relationships across the six PFTs and four nutrient treatments, consistent with the  
285 rationale used in past studies (Reich *et al.*, 2008; Xiang *et al.*, 2013). Standardized major axis (SMA)  
286 analysis was used to determine the best-fitting lines ( $\alpha = 0.05$ ) for the key [log-log trait-trait](#)  
287 relationships (Falster *et al.*, 2006; Warton *et al.*, 2006; Warton *et al.*, 2012) ~~and leaf traits~~. We  
288 tested for differences among nutrient [supply treatments](#) and PFT classes. ~~SMA, descriptive statistics~~  
289 ~~and filtering~~ [Data exploration, screening for outliers and SMA analysis was performed using the R](#)  
290 [language and environment were done in R](#) (R Development Core Team, 2015) ~~and the smatr~~  
291 [package \(Warton \*et al.\* 2012\)](#). [Multiple comparison tests employed in the smatr package are](#)  
292 [explained in Appendix D of Warton \*et al.\* \(2006\) – see SI Notes S1 for further details.](#) ~~Data are to~~  
293 ~~be~~ freely available and published on the Australian Research Data portal (~~doi to be inserted: here~~).

294

## 295 Results

296

### 297 Leaf trait variation

298 Across the entire dataset, there was large variation in each [of the selected structural, chemical](#)  
299 [composition and physiology](#) traits, reflecting the diverse range of species (Fig. ~~s 2 and 3~~). For all  
300 traits, there were significant differences among PFTs ( $P < 0.0001$ , Table 1). When averaged across  
301 all treatments, mean  $M_a$  ~~for each~~ [across](#) PFTs varied five-fold, with needle-leaf coniferous ([NLT](#))  
302 trees [having](#) exhibited ~~ing~~ the highest values ( $225 \pm 10 \text{ g m}^{-2}$ ) while  $C_4$ -grasses ([C4G](#)) showed the  
303 lowest  $M_a$  ( $39 \pm 2 \text{ g m}^{-2}$ ) (Fig. ~~2a,c~~, Table S2). ~~This pattern was also reflected in  $N_a$  ( $3.6 \pm 0.3 \text{ g m}^{-2}$  in~~  
304 ~~NIT and  $0.7 \pm 0.02 \text{ g m}^{-2}$  in C4G) and  $P_a$  ( $0.93 \pm 0.13 \text{ g m}^{-2}$  in NLT) with the average of broadleaved~~  
305 ~~shrubs and trees (BLT) being  $0.37 \pm 0.05 \text{ g m}^{-2}$  while grasses exhibited mean  $P_a$  values of  $0.18 \text{ g m}^{-2}$~~   
306 ~~(C3G) and  $0.13 \text{ g m}^{-2}$  (C4G) (Fig. 2b). Overall, woody species exhibited higher  $N_a$  than their~~  
307 ~~herbaceous counterparts, largely reflecting differences in  $M_a$  among PFTs and PFT-dependent~~  
308 ~~differences in  $N_m$  (Table S2). Highest  $P_a$  were again found in NIT ( $0.93 \pm 0.13 \text{ g m}^{-2}$ ) with the average~~  
309 ~~of broadleaved shrubs and trees being  $0.37 \pm 0.05 \text{ g m}^{-2}$ , while grasses exhibited mean  $P_a$  values of~~  
310  ~~$0.18 \text{ g m}^{-2}$  (C3G) and  $0.13 \text{ g m}^{-2}$  (C4G) (Fig. 2c). N:P ratios showed significant differences between~~  
311 ~~each treatment (Table 2) with N:P above 15 in broad-leaved trees (BLT), shrubs (S) and  $C_3$  grasses~~  
312 ~~(C3G); PFT differences are shown in (Table S2). Mass-based values of both nitrogen ( $N_m$ ) and~~

313 phosphorus ( $P_m$ ) were lowest in S and NLT and highest in C4G and C3H (Table S2, Fig. 2). Within  
314 each PFT, high and low nutrient treatment concentrations were reflected in the leaf traits  
315 summarised in Table S2 and Figure S1. Mean  $A_a$  values were similar across PFTs, ranging from 10.1  
316 to 15.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for C3G and S, respectively (Fig. 3a2d);  $A_m$  was highest in C4G and C3H and  
317 lowest for ~~NLT~~NLT (data not shown). Highest rates of  $R_{\text{Dark},a}$  were found for S and ~~BLT~~BLT (mean 1.13  
318  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) with the lowest means in C3G (0.49  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Fig. 3b2e). By contrast,  $R_{\text{Light},a}$  was  
319 highest in ~~NLT~~NLT and C4G (mean 0.95  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and lowest in C3G (0.44  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Fig. 3e2f,  
320 Table S2).

321

### 322 *Impacts of nutrient supply on mean values*

323 Table S2 shows mean values for each PFT both among and across treatments, while Table 1 shows  
324 the two-way ANOVA assessing main and interactive effects of PFT and treatment. Growth of plants  
325 on contrasting N and P supply resulted in significant changes in foliar N and P, both on a mass and  
326 area basis (Table 1). Although there were differences in leaf chemistry among PFTs (see above and  
327 Table S2), overall treatments receiving high N supply exhibited the highest  $N_m$  (24.5  $\text{mg g}^{-1}$ ,  
328 averaged across both  $H_N H_P$  and  $H_N L_P$ ; Table 2) compared to the low N treatments with an average  
329  $N_m$  of 15.9  $\text{mg g}^{-1}$ . Low P treatment reduced  $N_m$  and  $N_a$  with high N supply, whereas under low N  
330 supply, P supply did not impact on leaf N (Table 2, Fig. S1). The highest  $P_m$  were found in high P  
331 supply both for high N and low N conditions, with the lowest  $P_m$  found in high N-low P supply  
332 ( $H_N L_P$ ). Low P supply resulted in a decline in  $P_a$  on both high and low N supply. Interestingly, low N-  
333 grown plants exhibited higher  $P_m$  compared to high N-grown plants (Table 2, Fig. S1). For woody  
334 PFTs (i.e. ~~BLT~~BLT, ~~NLT~~NLT and S), there was no effect of low N on area-based soluble sugars (Table  
335 3). Under high N conditions, low P supply led to a 60% increase in starch and a 15% increase in total  
336 non-structural carbohydrates (TNC) per unit leaf area in the woody PFTs (Table 3 and SI Table S3).  
337 Whereas N and P supply had large effects on foliar chemistry, nutrient supply had no effect on  $M_a$ ,  
338 averaged across all PFTs (Tables 1 and 2, Fig. S1a). Moreover, correcting  $M_a$  values for  
339 accumulation of TNC did not alter this conclusion, when considering woody species alone. The  
340 stability of  $M_a$  suggests we can equally assess the effect of nutrient supply on metabolic traits on  
341 either an area- or mass-basis.

342 Nutrient supply had a marked effect on fluxes of area- and mass-based rates of  
343 photosynthesis and respiration (Tables 1 and 2). The highest rates of both  $A$  and  $R$  occurred in the

344 H<sub>N</sub>H<sub>P</sub> treatment and all other treatments exhibited similar, lower rates of  $A_a$  and  $A_m$ . -  $A_a$  and  $A_m$   
345 were reduced to a similar extent when either N or P were in low supply (reduced by 20% for  $A_a$ ) and  
346 with no further reduction when both N and P were in low supply (Table 2). Similarly, for  $R_{\text{Dark}}$  (both  
347 on area and mass bases) low N treatment resulted in significant declines (-27%), as did low P supply  
348 (-16%) compared to the H<sub>N</sub>H<sub>P</sub> treatment. However, unlike photosynthesis, L<sub>N</sub>L<sub>P</sub> supply resulted in  
349 further significant declines in  $R_{\text{Dark}}$  compared to when either N or P were in low supply (Table 2).  
350  $R_{\text{Light}}$  showed similar patterns as  $R_{\text{Dark}}$ , with lowest rates in L<sub>N</sub>L<sub>P</sub>. Compared to the H<sub>N</sub>H<sub>P</sub> treatment,  
351  $R_{\text{Light}}$  exhibited reduced rates in low N regardless of P supply (Table 2). Hence, there was no effect of  
352 P deficiency on both photosynthesis and respiration (in either the dark or light) in low N-grown  
353 plants (both L<sub>N</sub>H<sub>P</sub> and L<sub>N</sub>L<sub>P</sub>), with P deficiency only affecting fluxes in high N-grown plants (Table 2).  
354 When averaged across all PFTs, P photosynthetic N use efficiency ( $A_N$ ) was 11% enhanced  
355 when plants experienced low N supply (compared to H<sub>N</sub>H<sub>P</sub>). By contrast,  ~~$A_N$  was reduced by~~ low P  
356 supply only reduced average  $A_N$  in plants grown on high N (-20%) ~~but not when N was limiting~~  
357 (Table 2). For respiration, N and P deficiencies had little effect on N-based rates of  $R_{\text{Dark}}$  and  $R_{\text{Light}}$   
358 (Table 2). For both  $A$  and  $R$ , rates expressed on a foliar P basis were significantly higher in low P-  
359 grown plants, irrespective of N supply (Table 2), suggesting greater P-use efficiency under low P  
360 conditions.

361 N-deficiency had no effect on respiration:photosynthesis ratios (i.e. the inhibitory effect of  
362 low N on  $A$  and  $R$  was similar) when P was non-limiting (Table 2). By contrast, under P-deficient  
363 conditions, low N supply resulted in a slight decrease in the  $R_{\text{Dark}}:A$  and  $R_{\text{Light}}:A$  ratios, reflecting the  
364 significant decline in respiration alone (i.e. no change in  $A$ ) under L<sub>N</sub>L<sub>P</sub> compared to H<sub>N</sub>L<sub>P</sub> conditions  
365 (Table 2). Finally, nutrient availability had no effect on the  $R_{\text{Light}}:R_{\text{Dark}}$  ratio (Table 2), with the  
366 average the  $R_{\text{Light}}:R_{\text{Dark}}$  ratio among treatments being  $0.74 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $n = 710$ ). Thus, light  
367 inhibited leaf respiration by 26% across the entire dataset.

368 Table 1 also provided insights into whether the effect of N supply on each trait differed  
369 among PFTs. The absence of a significant interaction term indicated that all PFTs were similarly  
370 affected by availability of N and P for  $M_a$ ,  $A_a$  and  $R_{\text{Light},a}$  (Table 1). For  $R_{\text{Dark},a}$ , the effect of treatment  
371 did differ among PFTs (Table S2); in general, low N supply reduced  $R$  in ~~BIF~~BLT and S but not in  
372 ~~NT~~NLT or grasses. Growth on H<sub>N</sub>L<sub>P</sub> supply led to significantly lower  $R_{\text{Dark},a}$  in ~~BIF~~BLT alone, with no  
373 effect of P supply on other PFTs (Table S2). At low N, low P supply only reduced rates of  $R_{\text{Dark},a}$  in  
374 C3G. Taken together, these results point to a general pattern of low N supply having its greatest

375 impact on metabolic traits when P is non-limiting (thus supporting Hypothesis 1) and that the  
376 impact of nutrient supply is more commonly seen in broadleaved trees and shrubs than the other  
377 PFTs.

378

### 379 *Leaf trait relationships*

380 The above analyses point to increased rates of  $A_N$  under low N supply (irrespective of P supply), and  
381 reduced  $A_N$  when P is limiting (for high N plants only), suggesting nutrient-mediated shifts in  
382 relationships between photosynthesis and N. We explored whether such changes were associated  
383 with shifts in proportionality-coefficient (i.e. elevation of the log-log relationships) ~~elevation~~ and/or  
384 scaling-exponents (i.e. slope) ~~slopes~~ of the  $A_a$ - $N_a$  relationship (as hypothesized in Fig. 1) and other  
385 area-based trait relationships via standardized major axis (SMA) analyses.

386 Figure 4-3 shows bivariate relationships among  $N_a$ ,  $P_a$  and  $M_a$ , with the corresponding SMA  
387 results shown in Table 4. While substantial scatter occurred within each bivariate relationship,  
388 significant positive relationships were apparent for each separate nutrient combination. For the  $N_a$ -  
389  $M_a$  relationship, there were no significant differences in scaling-exponents ~~slopes~~ of the four  
390 treatments, but significant differences were found in the proportionality-coefficient ~~elevation~~ of  
391 the fitted lines (Fig. 4a3a, Table 4). Here,  $N_a$  at a given  $M_a$  was significantly higher in the  $H_N H_P$   
392 treatment, followed by  $H_N L_P$ , which in turn was higher than  $L_N H_P$  and  $L_N L_P$  relationships (i.e.  $H_N H_P >$   
393  $H_N L_P > L_N H_P = L_N L_P$ ). Thus, leaves consistently accumulated more leaf N for a given  $M_a$  under high N  
394 conditions, with P deficiency slightly decreasing how much N accumulated at given  $M_a$ . For the  $P_a$ -  
395  $M_a$  relationship (Fig. 3b), ~~there was a significant difference in slopes among the treatments because~~  
396  $L_N H_P$  had a steeper-greater scaling-exponent ~~slope~~ compared to the other treatments (Table 4);  
397 Figure 4b-3b also shows that  $P_a$  values at a given  $M_a$  were lower in the two low-P treatments than  
398 the two high-P treatments. When considering the  $N_a$ - $P_a$  relationship, SMA analyses revealed  
399 ~~different~~ ees among the scaling-exponents ~~slopes~~, with  $L_N H_P$  exhibiting a flatter  $N_a$ - $P_a$  response than  
400 the other treatments.  $N_a$  at a given  $P_a$  was highest in  $H_N L_P$  plants (Fig. 4c3c), followed by  $L_N L_P$  and  
401  $H_N H_P$  grown plants, which in turn were markedly higher than  $L_N H_P$  plants. Thus, the treatments  
402 differed in a manner consistent with N:P values (Table 2).

403 We now explore how the treatments affected bivariate relationships linking  $A_a$  to  $N_a$ ,  $P_a$  and  
404  $M_a$  (Fig. 54, Table 4). For  $A_a$ - $N_a$  relationships (Fig. 5a4a), ~~showed different slopes. S~~ similar growth  
405 on low N supply resulted in greater scaling-exponents ~~slopes~~ compared to high N grown plants,

406 irrespective of P supply (Table 4). This finding reflected two interesting outcomes. Firstly, for  
407 plants grown on high P supply, growth on low N ( $L_N H_P$ ) only increased  $A$  at a given  $N$  (i.e. increased  
408  $A_N$ ) in species with inherently high  $N_a$  values, with little effect of  $N$  supply on  $A_N$  in low  $N_a$  species.  
409 By contrast, when P supply was limiting, low N supply had little effect on  $A_N$  in high  $N_a$  species, but  
410 increased  $A_N$  in low  $N_a$  species. Thus, the effect of  $N$  supply on  $A_N$  differs among the contrasting  
411 species used in our study, and depends on whether P supply was limiting. ~~were exhibited by the~~  
412 ~~two low N treatments (i.e.  $L_N H_P$  &  $L_N L_P$ ) but plants grown on  $H_N L_P$  exhibited a much steeper slope~~  
413 ~~than the other treatments. Hence~~ Underpinning this observation was that, with high N availability,  
414 ~~growth on~~ low P supply reduced  $A_N$  in low  $N_a$  species, but had little effect on  $A_N$  in high  $N_a$  species.  
415 ~~had a marked inhibitory effect on photosynthesis, but when N was limiting, P availability had~~  
416 ~~no effect.~~ For  $A_a$ - $P_a$  relationships (Fig. 5b4b),  $H_N L_P$  grown plants exhibited a significantly steeper  
417 greater scaling-exponent ~~slope~~ than the other treatments (Table 4), with  $A_a$  at a given  $P_a$  differing  
418 between high and low N treatments. For the low P-grown plants, those provided with high N  
419 exhibited generally greater  $A_a$  at a given  $P_a$  than low N-grown plants (i.e. comparing green with  
420 yellow orange, and blue with red black lines symbols in Fig. 5b4b; note:  $A_a$ - $P_a$  relationship was not  
421 significant for  $L_N L_P$  plants). Finally, when considering the relationship between  $A_a$  and  $M_a$ , no  
422 relationship was found for high N-grown plants (Table 4), with a common relationship being shared  
423 by low N grown ( $L_N H_P$  and  $L_N L_P$ ) plants (grey line in Fig. 4c). Taken together, photosynthesis was  
424 reduced with low P supply in high N-grown plants with respect to the  $A_a$ - $N_a$  relationship, whereas  
425 ~~but~~ with low N supply ~~leading to reduced~~ rates of  $A_a$  for a given  $P_a$  were reduced, both in high and  
426 low P availability.

427 For relationships linking leaf respiration to other traits  $N_a$ ,  $P_a$  and mass-area  $M_a$ , we found that  
428 treatment had no effect on the scaling-exponents ~~slopes~~ of  $R_{Dark,a}$ - $N_a$ , but did alter the scaling-  
429 exponents ~~slopes~~ of the  $R_{Light,a}$ - $N_a$  relationship (Fig. 654, Table 4). For  $R_{Dark,a}$ - $N_a$ , plants grown on  
430 high N, P-deficiency resulted in lower  $R_{Dark,a}$  at a given  $N_a$  (i.e. lower proportionality-coefficient),  
431 particularly in plants where foliar N concentrations were low. By contrast, at low N supply,  
432 availability of P had no effect on  $R_{Dark,a}$  at a given  $N_a$  (Fig. 6a54ad); this was also true for the  $R_{Light,a}$ -  
433  $N_a$  relationship (Fig. 6d54dg). Thus, as was the case for photosynthesis, P availability only modifies  
434 the relationship between leaf respiration and  $N_a$  when N supply is not limiting.

435 Growth on low P resulted s exhibited in higher  $R_{Dark,a}$  and  $R_{Light,a}$  at a given  $P_a$ , irrespective of N  
436 supply (Fig. 6b54eb, eh). Moreover, limitations in the availability of N resulted in lower  $R_{Dark,a}$  and



437  $R_{\text{Light},a}$  at a given  $P_a$ ; this was true for plants grown on low and high P. Thus, relationships linking leaf  
438 respiration to  $P_a$  ~~are~~ were influenced not only by the availability of P, but also by N supply. Finally,  
439 whereas no significant relationships were found when considering  $R_{\text{Light},a}$  as a function of  $M_a$  (Fig.  
440 ~~6f54fi~~, Table 4),  $R_{\text{Dark},a}$ - $M_a$  relationships were significant for all four treatments (albeit with a low  $R^2$ ;  
441 Fig. ~~6e54fe~~, Table 4). Interestingly, expressing rates on a mass basis resulted in highly significant  
442  $R_{\text{Light},\text{em}}$ - $M_a$  (and  $R_{\text{Dark},\text{em}}$ - $M_a$ ) relationships (SI Fig. S2, Table S4). For  $R_{\text{Dark},a}$ - $M_a$  (Fig. 4f), nutrient  
443 treatment had no effect on relationship slopes, with elevations being ranked from highest to  
444 lowest:  $H_{\text{NH}_P} > H_{\text{NL}_P} = L_{\text{NH}_P} > L_{\text{NL}_P}$ . Thus, the general pattern was one of  $R_{\text{Dark},a}$  at a given  $M_a$   
445 decreasing as nutrient availability declined (Table 2).

446 Nutrient availability did not significantly affect rates of  $R_{\text{Dark},a}$  at a given  $A_a$  indicated via a  
447 common  $R_{\text{Dark},a}$ - $A_a$  relationship across all four treatments (shown by the common grey regression  
448 line in Fig. 7a65a). ~~While there were significant differences in scaling-exponents slopes~~ when  
449 considering  $R_{\text{Light},a}$ - $A_a$  (Fig. ~~7b65b~~, Table 4), the overall pattern was similar to that seen in  $R_{\text{Dark},a}$ - $A_a$   
450 ~~Figure 7a~~. On first inspection, the finding that  $R_{\text{Dark},a}$ - $A_a$  was unaffected by nutrient supply contrasts  
451 with the comparison of means shown in Table 2, where limitations in N supply reduced  $R_{\text{Dark},a}$ - $A_a$   
452 ratios in low P-grown plants. However, given the scattered nature of the bivariate plots and the  
453 slight differences in  $R_{\text{Dark},a}$  at a given  $A_a$  among the treatments (Fig. ~~765a~~), caution is needed when  
454 considering small changes in mean-alone data shown in Table 2. Thus, the most parsimonious  
455 conclusion is that nutrient supply had little or no effect on  $R_{\text{Dark},a}$ - $A_a$  ratios. This was also true for the  
456  $R_{\text{Light},a}$ - $R_{\text{Dark},a}$  relationships among four treatments, with nutrient supply having no effect (Fig.  
457 965ca; Table 44); consequently, the degree of light inhibition was unaffected by nutrient supply (~  
458 26% inhibition; Table 2).

459 While ~~nutrient supply had no significant effect on the slope in Figure 7a6a~~, there remained  
460 ~~considerable scatter in the overall  $R_{\text{Dark},a}$ - $A_a$  relationship. Thus, other factors distinct from nutrient~~  
461 ~~supply must be responsible for the scatter. Differences between PFTs woody and herbaceous~~  
462 ~~species showed is an obvious candidate via no significant difference in ~~slopes~~ scaling-exponents ( $P =$   
463 0.37) for  $R$ - $A$  relationships, ~~w~~ but significant differences in elevation (Table S5). W woody species  
464 exhibited ~~tinged~~ a higher proportionality-coefficient elevation (i.e. less negative y axis intercept)  
465 compared to herbaceous PFTs (Fig. S3a65d, Table S54). Hence, for a given  $A_a$ , woody species overall  
466 had higher dark respiration compared to herbaceous species, with  $R_{\text{Dark},a}$  at a given  $A_a$  being highest  
467 in shrubs and broadleaf trees followed by  $C_3$  herbs, while ~~needle-leaf~~ NLT trees,  $C_3$  and  $C_4$  grasses~~

468 exhibited the lowest  $R_{\text{Dark},a}$  at a given  $A_a$  (Fig. S3, Table S5). For  $R_{\text{Light},a}-A_a$  (Fig. 5e), there was no  
469 difference between herbs and woody species in the scaling-exponent ~~slope~~ or proportionality-  
470 coefficient ~~elevation of the  $R_{\text{Light},a}-A_a$  relationship~~ (but there was a shift moving along the same  
471 relationship); this is ~~in~~ contrast to  $R_{\text{Dark},a}-A_a$  (Fig. 65ed) where woody species exhibited a higher  
472 proportionality-coefficient than herbs. For the  $R_{\text{Light}}-R_{\text{Dark}}$  relationship, ~~h~~Herbaceous species  
473 ~~showed~~ exhibited a ~~higher~~ greater scaling-exponent ~~slope in the  $R_{\text{Light}}-R_{\text{Dark}}$  relationship~~ compared to  
474 woody species (Fig. 56f, Table S5). ~~with~~ The lowest degree of light inhibition of leaf respiration  
475 was found in C4G (as shown by the highest  $R_{\text{Light}}:R_{\text{Dark}}$  slope, Table S5) and the highest degree of  
476 light inhibition found in NLT (lowest  $R_{\text{Light}}:R_{\text{Dark}}$  scaling-exponent ~~slope~~).

477  
478 ~~with  $R_{\text{Dark},a}$  at a given  $A_a$  being highest in shrubs and broadleaf trees followed by  $C_3$  herbs,~~  
479 ~~with needle leaf trees,  $C_3$  and  $C_4$  grasses exhibiting the lowest  $R_{\text{Dark},a}$  at a given  $A_a$  (Fig. 8, Table 5).~~  
480 ~~SMA analyses on woody and herbaceous PFTs indicated no significant difference in slopes ( $P=0.37$ )~~  
481 ~~but different elevations, with woody species exhibiting a higher elevation (i.e. less negative y-axis~~  
482 ~~intercept) compared to herbaceous PFTs (Fig. S3a, Table 5). Hence, for a given  $A_a$ , woody species~~  
483 ~~had higher dark respiration compared to herbaceous species.~~

484 ~~For  $R_{\text{Light},a}-A_a$ , there was less consistency in the bivariate relationships among PFTs (Table 5).~~  
485 ~~When combining PFTs into woody and herbaceous categories, then there was no difference in slope~~  
486 ~~or intercept of the  $R_{\text{Light},a}-A_a$  relationship (but there was a shift moving along the same relationship)~~  
487 ~~in contrast to  $R_{\text{Dark},a}-A_a$  (Fig. S3b). Lastly, Figure 9 shows  $R_{\text{Light},a}-R_{\text{Dark},a}$  relationships among four~~  
488 ~~treatments, with nutrient supply having no effect (Fig. 9a, Table 4); consequently, the degree of~~  
489 ~~light inhibition was unaffected by nutrient supply ( $\sim 26\%$  inhibition, Table 2). However, the lowest~~  
490 ~~degree of light inhibition found in C4G (highest  $R_{\text{Light}}:R_{\text{Dark}}$  slope, Table 5) and the highest degree of~~  
491 ~~light inhibition found in NITNLT (lowest  $R_{\text{Light}}:R_{\text{Dark}}$  slope). Combining PFT into woody and~~  
492 ~~herbaceous species indicated a higher  $R_{\text{Light}}:R_{\text{Dark}}$  slope in herbaceous compared to woody species~~  
493 ~~(Fig. 9b, Table 5).~~

494  
495

## 496 Discussion

497

498 ~~Study rationale and~~ Extent of nutrient limitations

499 ~~Nitrogen and phosphorus exert strong and fundamental controls on metabolic performance of~~

500 ~~leaves, as seen across both natural and fertilized soil gradients (Reich & Schoettle, 1988; Cordell et~~

501 ~~al., 2001; Whitehead et al., 2005). However, given strong tendencies for leaf N and P to co-vary in~~

502 ~~nature (Sterner & Elser, 2002; Reich & Oleksyn, 2004; Ågren et al., 2012), it is difficult to isolate the~~

503 ~~effects of low P from low N in explaining broad patterns of variation in relationships between~~

504 ~~metabolism and leaf nutrients. This is especially so in field studies comparing species from among~~

505 ~~biomes and among sites within individual biomes (Kattge et al., 2009; Reich et al., 2009; Domingues~~

506 ~~et al., 2015; Bahar et al., 2016). Given these challenges, we used a factorial design to isolate the~~

507 ~~individual and combined effects of N and P availability on a range of leaf traits associated with~~

508 ~~photosynthesis and respiration. Our findings show that both N and P supply can mediate leaf trait~~

509 ~~trait relationships, suggesting that the next generation of Earth System Models should consider~~

510 ~~how limitations in N and P supply in individual biomes may affect trait relationships (Goll et al.,~~

511 ~~2012).~~

512 In setting up our experiment, there was a risk that the low P and N treatments would not result in

513 P- and N-deficient phenotypes (in terms of lower metabolic rates) needed to test our working

514 hypotheses.  ~~Past studies have shown that total P concentrations are strongly influenced by storage~~

515 ~~of P in vacuoles (Foyer & Spencer, 1986; Raghothama, 1999) and this storage pool can buffer~~

516 ~~cytosolic P.~~ Hence measured differences in foliar leaf P may not showing a metabolically different

517 phenotypes despite measured differences in foliar P. Similarly, the fact that investment of leaf N in

518 metabolism is a plastic trait – for example, increasing as a proportion of total N when N supply is

519 limiting (Cheng & Fuchigami, 2000) - means that low N treatments will not ~~on't~~ necessarily result in

520 lower metabolic rates. However, we found rates of  $A_a$  and  $R_a$  that were lower in low P-grown plants

521 (compared to  $H_N H_P$ ), demonstrating that the supply of P was sufficiently low to create P-deficient

522 phenotypes (Table 2). Moreover, as reported previously ~~for a range of plant species~~ (Hammond &

523 White, 2008), starch accumulation occurred in low P-grown plants of the woody PFTs (Table 3),

524 consistent with a P-deficient phenotype. The low N treatment also resulted in reductions in  $A_a$  and

525  $R_a$ , and reduced mass-based concentrations of soluble sugars in woody PFTs (Table 3),

526 demonstrating that N-deficient phenotypes were achieved. Importantly, the impact of the low P-

527 and N treatments on metabolic rates and ~~area-based~~ log-log trait-trait relationships depended on  
528 the extent to which supply of each nutrient was limiting. ~~Our study provides the first multi-PFT~~  
529 ~~comparison assessing nutrient impacts on leaf trait relationships under controlled environment~~  
530 ~~conditions.~~

531

532 *How does P mediate A-R-N relationships, and is the impact of P independent of N supply?*

533 ~~Past work has shown that rates of A at a given N are often lower at sites with low P availability~~  
534 ~~(Meir et al., 2002; Kattge et al., 2009; Reich et al., 2009; Domingues et al., 2010; Bahar et al., 2016).~~

535 Based on knowledge of how P deficiencies affect leaf metabolism (Warren & Adams, 2002; Plaxton  
536 & Tran, 2011; Bloomfield et al., 2014; Ellsworth et al., 2015) and studies comparing trait

537 relationships of plants growing on soils with contrasting P levels (Kattge et al., 2009; Reich et al.,  
538 2009), we predicted that P deficiency would reduce the proportionality-coefficient of log-log A-N,

539  $R_{\text{Dark-N}}$  and  $R_{\text{Light-N}}$  relationships, but with no change in the scaling-exponent of the log-log

540 relationships shallower A-N slopes in P-limited plants (Fig. 1). However, ~~contrary to the~~

541 ~~hypothesized relationships outlined in Fig. 1, inspection of Figure 4a shows that growth on limiting~~  
542 ~~P supply did not result in a decreased slope in the  $A_a$ - $N_a$  relationship; rather,~~ when plants were

543 grown on high N, low P resulted in a significant increase in scaling-exponent ~~slope~~ of  $A_a$ - $N_a$

544 relationship, reflecting a decrease in  $A_a$  in low  $N_a$  species with low, with P deficiency having little or

545 no effect on  $A_a$  in species with high  $N_a$ . The same was true for the  $R_{\text{Dark},a}$ - $N_a$  relationship (Fig. 4d);

546 ~~consistent with Rowland et al. (2016) reporting higher rates of  $R_{\text{Dark}}$  for a given N in low nutrient~~

547 ~~soils. While the reasons for this increase in scaling-exponent under low P-high N conditions remain~~

548 ~~uncertain, the results highlight how contrasting species differing in leaf structure/chemistry differ in~~

549 ~~their responses to P deficiency~~ Stimulation ~~of  $A_a$  (or  $R_{\text{Dark},a}$ ) under low P conditions (when N is~~

550 ~~not limiting but not under N-limiting conditions) might reflect the fact that N supply can play a role~~

551 ~~in stimulating P uptake in low P soils (Treseder & Vitousek, 2001).~~

552 ~~One of our working hypotheses was that the effect of~~ We expected that low P supply on

553 metabolic rates would be greatest in plants supplied with high N, based on past observations on the

554 stimulatory effect of P addition being greatest when N is non-limiting (e.g. Aerts et al., 1992; Meir

555 et al., 2001). ~~We found~~ Our results support that ~~l~~ low P supply had minimal effects on metabolic

556 rates when N was limiting (Table 2) - both for photosynthesis and respiration - while the inhibitory

557 effect of low P was greatest when N was not limiting. ~~These results are consistent with Liebig's law~~

558 ~~where the most limiting element has the greatest impact.~~ A phosphorus P supply-mediated effect  
559 on metabolic relationships with N has been reported in several tropical studies (Reich & Walters,  
560 1994; Denton *et al.*, 2007; Meir *et al.*, 2007; Mercado *et al.*, 2011; Quesada *et al.*, 2012), which  
561 typically have lower P availability compared to N supply reflected in high N:P ratios.

562 Interestingly, P had *no* effect at all on the log-log  $A_m$ - $N_m$  relationship irrespective of N supply  
563 (Fig. S2). ~~The latter result contrasts with Reich *et al.* (2009), where~~ log-log  $A_m$ - $N_m$  relationships  
564 differed among plants growing at P-rich and P-deficient sites from field studies representing a range  
565 of nutrient availability, soil properties and mycorrhizal associations, thermal environment, water  
566 availability and species composition. In controlled-environment conditions ~~our study,~~ we compared  
567 the effect of low P supply on a common set of species ~~grown under common, controlled~~  
568 ~~environment conditions,~~ suggesting that contrasting relationships (via different ~~slopes~~ scaling-  
569 exponents or ~~intercepts~~ proportionality-coefficients ~~elevation~~) from different sites and biomes may  
570 include variation other than just soil nutrient supply. Given this, care is needed when interpreting  
571 the direct role of nutrient availability on  $A_m$ - $N_m$  relationship slopes across field sites.

#### 572 *Mechanisms underpinning changes in photosynthetic N use efficiency.*

573 Consistent with our working hypotheses (Fig. 1), we found that overall mean values of leaf-level N-  
574 use efficiency of photosynthesis ( $A_N$ ) ~~was~~ were enhanced by low N supply, both under low and high  
575 P supply (Table 2, ~~Fig. 5a4a~~). Cheng & Fuchigami (2000) found that  $A_N$  of apple leaves also  
576 increased with decreasing N supply. However, not all studies have reported this response, with  
577 Warren *et al.* (2003), Hikosaka (2010) and Pons & Westbeek (2004) all reporting little effect of N  
578 supply on photosynthetic N use efficiency. Our data suggest that while low N treatment increased  
579  $A_N$  of some species – particularly species that exhibit inherently low  $N_m$  values (Fig. S2) – low N  
580 treatment had little or no effect on other species (e.g. high  $N_m$  species). Given that species with  
581 inherently low  $N_m$  tend to also be slow-growing and/or found in less productive environments  
582 (Wright *et al.*, 2004), this suggests that plasticity of  $A_N$  in response to variation in N supply may  
583 systematically vary among species adapted to contrasting environments. The increase in  $A_N$  in low  
584 N supply can occur via several possible ways (Hikosaka, 2004), such as: (1) greater fraction of leaf N  
585 allocated to photosynthetic processes, particularly the relative investment of leaf N in Rubisco  
586 (Evans & Seeman, 1989; Poorter & Evans, 1998; Warren *et al.*, 2003); (2) increase in the activation  
587 state of Rubisco (Stitt & Schulze, 1994; Warren *et al.*, 2000); (3) reduction of N investment in

589 defence compounds, cell walls and/or nucleic acids (Chapin & Kedrowski, 1983; Onada *et al.*, 2004;  
590 Harrison *et al.*, 2009); or (4) a decrease in inorganic N accumulation (e.g.  $\text{NO}_3^-$  in vacuoles) to  
591 maintain organic N investment in metabolism. Similar factors are also likely to play a role in  
592 decreased  $A_N$  exhibited by high N-grown plants with limiting P supply (Table 2) (Warren & Adams,  
593 2002; Bloomfield *et al.*, 2014). Indeed, there was a negative relationship in all PFTs between N-use  
594 efficiency and N:P ratio with more P-limited plants (high N:P ratio) exhibiting lower nitrogen-use  
595 efficiencies for both  $A_N$  and  $R_{\text{Dark},N}$  (Fig. S4).

596

### 597 *Plant functional types and bivariate relationships*

598 In our study, 37 species from six PFTs were used ~~to create a spectrum of leaf traits varying in~~  
599 ~~structure, chemistry and function, which enabled us~~ to assess whether the effect of nutrient supply  
600 on leaf traits differed among the PFTs. ~~Table 1 showed that for all leaf chemistry traits, t~~The  
601 magnitude of nutrient-mediated changes in leaf traits differed among the PFTs (Table 1). ~~Proteoid~~  
602 species exhibited steeper  $A_a$ - $N_a$  relationships when P was low compared to broadleaved trees but  
603 not when P was high (Fig. S5). However, the distinct PFTs exhibited relatively similar responses to  
604 treatments when considering structural (i.e.  $M_a$ ) and metabolic traits (particularly  $A_a$  and  $R_{\text{Light},a}$ ).  
605 Thus, despite enormous differences in leaf life span, leaf nutrients and structural components  
606 among the PFTs, generally speaking all PFTs exhibited similar metabolic responses to nutrient  
607 availability, consistent with previous field studies (Reich *et al.*, 2008).

608 ~~While n~~Nutrient supply had no effect on the ratio between leaf dark respiration and  
609 photosynthesis (i.e. ~~no significant difference in slope~~ a common relationship across all four  
610 treatments in Fig. 5a). However, ~~our finding of there was~~ PFT-dependent variation in  $R_{\text{Dark},a}$ - $A_a$   
611 scaling (Fig. ~~85d6~~, Fig. S3), with PFTs differing in the proportional-coefficients of relationships that  
612 shared common scaling-exponents. This contrasts with recent field-based comparisons of PFTs in  
613 respiration-photosynthesis relationships (Atkin *et al.*, 2015), where there was little evidence of  $C_3$   
614 herbs/grasses, broad leaved trees, shrubs and needle-leaved trees differing in rates of  $R_{\text{Dark},a}$  at a  
615 given  $A_a$ . However, such comparisons may be strongly influenced by a range of environmental  
616 factors that may mask underlying PFT-dependent patterns. Our study, conducted under controlled  
617 environment conditions provided strong evidence of PFT-mediated variations in the respiration:  
618 photosynthesis ratio in plants driven by differences between woody and herbaceous species.  
619 Future work will be needed to determine what underlying factors (e.g. respiratory costs associated

620 with protein turnover, phloem loading and maintenance of ion gradients in leaf cells; Lambers,  
621 1985; Noguchi & Yoshida, 2008) are responsible for this PFT-dependent variation in  $R_{\text{Dark,a-Aa}}$   
622 scaling.

623

#### 624 *R<sub>Light</sub> versus R<sub>Dark</sub>*

625 One of the objectives of our study was to assess whether nutrient supply affected the degree of  
626 light inhibition of non-photorespiratory mitochondrial CO<sub>2</sub> release ([see SI Notes S2 for further](#)  
627 [details](#)). Past work has reported that  $R_{\text{Light}}$  is often lower than  $R_{\text{Dark}}$ , with the degree of inhibition  
628 being highly variable (Griffin & Heskell, 2013) but typically around 30% (Crous *et al.*, 2012;  
629 Weerasinghe *et al.*, 2014). In leaves, respiratory energy is needed for assimilation of N and protein  
630 turnover, as well as for supporting the ATP demands from photosynthesis (Krömer, 1995). Given  
631 that N deficiency may limit such demands for respiratory products, one possibility was that the  
632 degree of inhibition could be linked to nutrient supply, with low N potentially affecting factors such  
633 as demand for TCA cycle intermediates and photorespiratory capacity. [In our study, we found that](#)  
634 ~~(Ayub *et al.*, 2014)~~ [However, as shown in Fig. 96c, across a broad range of species,](#) nutrient supply  
635 had no effect on the relationship between  $R_{\text{Dark}}$  and  $R_{\text{Light}}$  (Fig. 5c), with the overall degree of light  
636 inhibition being 26% across all treatments and species. Interestingly, the degree of light inhibition  
637 was generally less in herbaceous species compared to woody species (Fig. 9b6f), a result that merits  
638 testing in a broader set of species.

639

#### 640 *Conclusions*

641 ~~Our study assessed the impact of P limitations both under high and low N conditions, with the~~  
642 ~~results leading to the following insights: Our findings show that both N- and P supply can mediate~~  
643 ~~leaf trait-trait relationships, suggesting that the next generation of Earth System Models should~~  
644 ~~consider how limitations in N and P supply availability in individual biomes may affect trait~~  
645 ~~relationships (Goll *et al.*, 2012).~~  
646 ~~Linking plant metabolism to leaf nutrients, both A-N and R-N relationships were only affected by~~  
647 ~~low P when N availability was high. Low P supply did not have an effect on the slope of A-N and R-N~~  
648 ~~relationships when N was limiting. Thus, P availability only modified the relationship between leaf R~~  
649 ~~and N when N supply was not limiting (high N). Our findings do not support the hypothesized~~  
650 ~~responses shown in Figure 1; rather, rates of leaf A and R at a given leaf N were dependent on P~~

651 supply in species with inherently low  $N_a$  values but not in those with inherently high  $N_a$  values.  
652 Further work assessing patterns of N and P allocation will be needed to address what factors are  
653 responsible for these observations. Interestingly, our results also did not support Hypothesis 4,  
654 with ~~This finding supports our H.2 and is consistent with results from field-based studies (especially~~  
655 ~~in tropical ecosystems with high N:P ratios). Whereas less P is needed to meet metabolic demands~~  
656 ~~in N-deficient plants, in high N-grown plants, the effect of low P supply may be underpinned by~~  
657 ~~insufficient P allocated to metabolic processes or the extent to which P supply affect N allocation in~~  
658 ~~the plant.~~ [KC4]  
659 ~~In A-P and R-P relationships, low P reduced A and R for a given  $P_a$  compared to high P, supporting~~  
660 ~~the first part of H.1 and H.3, but low N supply reduced A and R rates both in high and low P~~  
661 ~~availability. In contrast to H.4, Photosynthesis rates were reduced under low P supply in high N-~~  
662 ~~grown plants in A-N and R-N relationships. By contrast, P supply had no effect at a given  $N_a$  when N~~  
663 ~~supply was limiting. Low N supply led to reduced A and R, both in high and low P supply. Thus, P~~  
664 ~~availability only modified the relationship between leaf R and N when N supply was not limiting~~  
665 ~~(high N).~~  
666 ~~Nutrient supply had having no effect on the ratio between  $R_{Dark}$  and  $A_a$ , although woody species~~  
667 ~~had a higher respiration rates for a given A compared to herbaceous species. This~~ The fact that  
668 coupling between respiration and photosynthesis remained constant suggests that deficiencies in  
669 nutrient availability have similar inhibitory effects on the capacity of processes. ~~suggests that~~  
670 ~~respiration proportionally declined with photosynthesis across species and nutrient treatments.~~  
671 Lastly Further, light inhibition of respiration was constant across nutrient treatments (26% on  
672 average) – again not supporting our working hypothesis - but with lower degrees of light inhibition  
673 in herbaceous than in woody species. Both findings have relevance to ecosystem models seeking to  
674 predict future rates of carbon exchange, as the degree of light inhibition can strongly influence  
675 ecosystem respiration estimates (Wohlfahrt *et al.*, 2005; Wehr *et al.*, 2016). Finally, the fact that  
676 both N- and P-supply can mediate leaf trait-trait relationships suggests that the next generation of  
677 Earth System Models may need to consider how limitations in N and P availability affect trait-trait  
678 relationships used to predict large scale carbon fluxes.  
679 Nutrient supply had no effect on the ratio between  $R_{Light}$  and  $R_{Dark}$ , with a light inhibition across  
680 treatments of 26%. Interestingly, our results point to a lower degree of light inhibition in  
681 herbaceous than in woody species.



682

683

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690

691 **Author contributions**

692 O.K.A., K.L.G., M.H.T., P.M. and K.Y.C. designed the study. K.Y.C., O.S.S., J.Z. and A.C.A.N. collected  
693 the [gas exchange](#) data [while O.S.S. ~~also~~ conducted leaf nutrient analyses](#). K.Y.C. led data processing,  
694 analyses and writing with substantial contributions from O.K.A. and K.J.B.. All authors made  
695 conceptual contributions throughout the manuscript development.

696

697

698 **References**

699

- 700 **Aerts R, Wall NB, Malmer N. 1992.** Growth-limiting nutrients in Sphagnum-dominated bogs subject to low  
701 and high atmospheric nitrogen supply. *Journal of Ecology* **80**: 131-140.
- 702 **Allen SE. 1974.** *Chemical Analysis of Ecological Materials*. Oxford: Blackwell Scientific Publications.
- 703 **Atkin OK, Bloomfield KJ, Reich PB, Tjoelker MG, Asner GP, Bonal D, Bönisch G, Bradford MG, Cernusak LA,**  
704 **Cosio EG et al. 2015.** Global variability in leaf respiration in relation to climate, plant functional types  
705 and leaf traits. *New Phytologist* **206**: 614-636.
- 706 **Atkin OK, Evans JR, Siebke K. 1998.** Relationship between the inhibition of leaf respiration by light and  
707 enhancement of leaf dark respiration following light treatment. *Australian Journal of Plant*  
708 *Physiology* **25**: 437-443.
- 709 **Atkin OK, Turnbull MH, Zaragoza-Castells J, Fyllas NM, Lloyd J, Meir P, Griffin KL. 2013.** Light inhibition of  
710 leaf respiration as soil fertility declines along a post-glacial chronosequence in New Zealand: an  
711 analysis using the Kok method. *Plant and Soil* **367**: 163-182.
- 712 **Atkinson LJ, Hellicar MA, Fitter AH, Atkin OK. 2007.** Impact of temperature on the relationship between  
713 respiration and nitrogen concentration in roots: an analysis of scaling relationships, Q<sub>10</sub> values and  
714 thermal acclimation ratios. *New Phytologist* **173**: 110-120.
- 715 **Ayub G, Smith RA, Tissue DT, Atkin OK. 2011.** Impacts of drought on leaf respiration in darkness and light in  
716 *Eucalyptus saligna* exposed to industrial-age atmospheric CO<sub>2</sub> and growth temperature. *New*  
717 *Phytologist* **190**: 1003-1018.
- 718 **Bahar NH, Ishida FY, Weerasinghe LK, Guerrieri R, O'Sullivan OS, Bloomfield KJ, Asner GP, Martin RE, Lloyd**  
719 **J, Malhi Y et al. 2016.** Leaf-level photosynthetic capacity in lowland Amazonian and high-elevation  
720 Andean tropical moist forests of Peru. *New Phytologist Online early (doi: 10.1111/nph.14079)*.
- 721 **Bloomfield KJ, Farquhar GD, Lloyd J. 2014.** Photosynthesis–nitrogen relationships in tropical forest tree  
722 species as affected by soil phosphorus availability: a controlled environment study. *Functional Plant*  
723 *Biology* **41** 820-832.
- 724 **Campbell CD, Sage RF. 2006.** Interactions between the effects of atmospheric CO<sub>2</sub> content and P nutrition on  
725 photosynthesis in white lupin (*Lupinus albus* L.). *Plant, Cell and Environment* **29**: 844-853.
- 726 **Chapin FS, Kedrowski RA. 1983.** Seasonal changes in nitrogen and phosphorus fractions and autumn  
727 retranslocation in evergreen and deciduous Taiga trees. *Ecology* **64**: 376-391.
- 728 **Cheng L, Fuchigami LH. 2000.** Rubisco activation state decreases with increasing nitrogen content in apple  
729 leaves. *Journal of Experimental Botany* **51**: 1687-1694.
- 730 **Clark DB, Mercado LM, Sitch S, Jones CD, Gedney N, Best MJ, Pryor M, Rooney GG, Essery RLH, Blyth E et**  
731 **al. 2011.** The Joint UK Land Environment Simulator (JULES), model description - Part 2: Carbon fluxes  
732 and vegetation dynamics. *Geoscientific Model Development* **4**: 701-722.
- 733 **Crous KY, Zaragoza-Castells J, Ellsworth DS, Duursma RA, LÖW M, Tissue DT, Atkin OK. 2012.** Light  
734 inhibition of leaf respiration in field-grown *Eucalyptus saligna* in whole-tree chambers under  
735 elevated atmospheric CO<sub>2</sub> and summer drought. *Plant, Cell & Environment* **35**: 966-981.
- 736 **Denton MD, Veneklaas EJ, Freimoser FM, Lambers H. 2007.** Banksia species (Proteaceae) from severely  
737 phosphorus-impooverished soils exhibit extreme efficiency in the use and re-mobilization of  
738 phosphorus. *Plant Cell and Environment* **30**: 1557-1565.
- 739 **Domingues FT, Ishida FY, Feldpausch T, Grace J, Meir P, Saiz G, Sene O, Schrodte F, Sonké B, Taedoumg H et**  
740 **al. 2015.** Biome-specific effects of nitrogen and phosphorus on the photosynthetic characteristics of  
741 trees at a forest-savanna boundary in Cameroon. *Oecologia* **178**: 659-672.
- 742 **Edwards EJ, McCaffery S, Evans JR. 2006.** Phosphorus availability and elevated CO<sub>2</sub> affect biological nitrogen  
743 fixation and nutrient fluxes in a clover-dominated sward. *New Phytologist* **169**: 157-167.
- 744 **Ellsworth DS, Crous KY, Lambers H, Cooke J. 2015.** Phosphorus recycling in photorespiration maintains high  
745 photosynthetic capacity in woody species. *Plant, Cell & Environment* **38**: 1142-1156.
- 746 **Evans JR. 1989.** Photosynthesis and nitrogen relationships in leaves of C<sub>3</sub> plants. *Oecologia* **78**: 9-19.

747 **Evans JR, Seeman JR 1989.** The allocation of protein nitrogen in the photosynthetic apparatus: costs,  
748 consequences and control. In: Briggs W ed. *Photosynthesis*. New York: Alan R. Liss, 183-205.

749 **Evans JR, Terashima I. 1988.** Photosynthetic characteristics of spinach leaves grown with different nitrogen  
750 treatments. *Plant Cell Physiology* **29**: 157-165.

751 **Falster DS, Warton DI, Wright IJ. 2006.** SMATR: Standardised major axis tests and routines, version 2.0. .

752 **Field CB, Mooney HA 1986.** The photosynthetic-nitrogen relationship in wild plants. In: Givnish T ed. *On the*  
753 *economy of form and function*. Cambridge, UK: Cambridge University Press, 22-55.

754 **Foyer CH, Spencer C. 1986.** The relationship between phosphate status and photosynthesis in leaves. Effects  
755 on intracellular orthophosphate distribution, photosynthesis and assimilate partitioning. *Planta* **167**:  
756 369-375.

757 **Fyllas NM, Patino S, Baker TR, Bielefeld Nardoto G, Martinelli LA, Quesada CA, Paiva R, Schwarz M, Horna**  
758 **V, Mercado LM et al. 2009.** Basin-wide variations in foliar properties of Amazonian forest:  
759 phylogeny, soils and climate. *Biogeosciences* **6**: 2677–2708.

760 **Gonzalez-Meler MA, Giles L, Thomas RB, Siedow JN. 2001.** Metabolic regulation of leaf respiration and  
761 alternative pathway activity in response to phosphate supply. *Plant Cell and Environment* **24**: 205-  
762 215.

763 **Griffin KL, Heskell M. 2013.** Breaking the cycle: how light, CO<sub>2</sub> and O<sub>2</sub> affect plant respiration. *Plant Cell and*  
764 *Environment* **36**: 498-500.

765 **Hammond JP, White PJ. 2008.** Sucrose transport in the phloem: integrating root responses to phosphorus  
766 starvation. *Journal of Experimental Botany* **59**: 93-109.

767 **Harrison MT, Edwards EJ, Farquhar GD, Nicotra AB, Evans JR. 2009.** Nitrogen in cell walls of sclerophyllous  
768 leaves accounts for little of the variation in photosynthetic nitrogen-use efficiency. *Plant Cell and*  
769 *Environment* **32**: 259-270.

770 **Heskell M, Greaves H, Kornfeld A, Gough L, Atkin OK, Turnbull MH, Shaver G, Griffin KL. 2013.** Differential  
771 physiological responses to environmental change promote woody shrub expansion. *Ecology and*  
772 *Evolution* **3**: 1149-1162.

773 **Heskell MA, Bitterman D, Atkin OK, Turnbull MH, Griffin KL. 2014.** Seasonality of foliar respiration in two  
774 dominant plant species from the Arctic tundra: response to long-term warming and short-term  
775 temperature variability. *Functional Plant Biology* **41**: 287-300.

776 **Hikosaka K. 2004.** Interspecific difference in the photosynthesis-nitrogen relationship: patterns,  
777 physiological causes, and ecological importance. *Journal of Plant Research* **117**: 481-494.

778 **Hikosaka K. 2010.** Mechanisms underlying interspecific variation in photosynthetic capacity across wild plant  
779 species. *Plant Biotechnology* **27**: 223-229.

780 **Hoagland DR, Arnon DI. 1950.** *The water-culture method for growing plants without soil*. Berkeley, Calif.:  
781 College of Agriculture, University of California.

782 **John MK. 1970.** Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil*  
783 *Science* **109**: 214-220.

784 **Kattge J, Díaz S, Lavorel S, Prentice IC, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright IJ et**  
785 **al. 2011.** TRY – a global database of plant traits. *Global Change Biology* **17**: 2905–2935.

786 **Kattge J, Knorr W, Raddatz T, Wirth C. 2009.** Quantifying photosynthetic capacity and its relationship to leaf  
787 nitrogen content for global-scale terrestrial biosphere models. *Global Change Biology* **15**: 976-991.

788 **Kirschbaum MUF, Farquhar GD. 1987.** Investigation of the CO<sub>2</sub> dependence of quantum yield and  
789 respiration in *Eucalyptus pauciflora* *Plant Physiology* **83**: 1032-1036.

790 **Kok B. 1948.** A critical consideration of the quantum yield of *Chlorella*-photosynthesis. *Enzymologia* **13**: 1-56.

791 **Krömer S. 1995.** Respiration during photosynthesis. *Annual Review of Plant Physiology & Plant Molecular*  
792 *Biology* **46**: 45-70.

793 **Lambers H 1985.** Respiration in intact plants and tissues: its regulation and dependence on environmental  
794 factors, metabolism and invaded organisms. *Encyclopedia of Plant Physiology. Volume 18, (Douce, R.*  
795 *& Day, D. A.)*. New York: Springer-Verlag, 417-473.

796 **Lambers H, Brundrett MC, Raven JA, Hopper SD. 2010.** Plant mineral nutrition in ancient landscapes: high  
797 plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant*  
798 *and Soil* **334**: 11-31.

799 **Leggett JE. 1971.** Growth and nutrient uptake by soybean plants in nutrient solutions of graded  
800 concentrations. *Plant Physiol* **48**: 457-460.

801 **Loveys BR, Atkinson LJ, Sherlock DJ, Roberts RL, Fitter AH, Atkin OK. 2003.** Thermal acclimation of leaf and  
802 root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Global*  
803 *Change Biology* **9**: 895-910.

804 **Makino A, Nakano H, Mae T. 1994.** Responses of ribulose-1,5-bisphosphate carboxylase, cytochrome-*f*, and  
805 sucrose synthesis enzymes in rice leaves to leaf nitrogen and their relationships to photosynthesis.  
806 *Plant Physiology* **105**: 173-179.

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

809 **Meir P, Levy PE, Grace J, Jarvis PG. 2007.** Photosynthetic parameters from two contrasting woody  
810 vegetation types in West Africa. *Plant Ecology* **192**: 277-287.

811 **Mercado LM, Lloyd J, Dolman AJ, Sitch S, Patino S. 2011.** Modelling basin-wide variations in Amazon forest  
812 productivity - Part 1: Model calibration, evaluation and upscaling functions for canopy  
813 photosynthesis (vol 6, pg 1247, 2009). *Biogeosciences* **8**: 653-656.

814 **Noguchi K, Yoshida K. 2008.** Interaction between photosynthesis and respiration in illuminated leaves.  
815 *Mitochondrion* **8**: 87-99.

816 **Noguchi KO, Terashima ICHI. 2006.** Responses of spinach leaf mitochondria to low N availability. *Plant, Cell*  
817 *and Environment* **29**: 710-719.

818 **Oleson KW, Lawrence DM, Bonan GB, Drewniak B, Huang M, Kovan CD, Levis S, Li F, Riley WJ, Subin ZM et**  
819 **al. 2013.** Technical Description of version 4.5 of the Community Land Model (CLM). In Research NCF A.  
820 *NCAR Technical Note NCAR/TN-503+STR*. Boulder, CO. 420.

821 **Onada Y, Hikosaka K, Hirose T. 2004.** Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-  
822 use efficiency. *Functional Ecology* **18**: 419-425.

823 **Pärnik T, Ivanova H, Keerberg O. 2007.** Photorespiratory and respiratory decarboxylations in leaves of C<sub>3</sub>  
824 plants under different CO<sub>2</sub> concentrations and irradiances. *Plant, Cell & Environment* **30**: 1535-1544.

825 **Plaxton WC, Podesta FE. 2006.** The functional organization and control of plant respiration *Critical Reviews*  
826 *in Plant Sciences* **25**: 159-198.

827 **Plaxton WC, Tran HT. 2011.** Metabolic adaptations of phosphate-starved plants. *Plant Physiology* **156**: 1006-  
828 1015.

829 **Pons TL, Van Der Werf A, Lambers H 1994.** Photosynthetic nitrogen use efficiency of inherently slow- and  
830 fast-growing species: possible explanations for observed differences. In: Roy J, Garnier E eds. *A*  
831 *Whole Plant Perspective on Carbon-Nitrogen Interactions*. Dordrecht, The Netherlands: SPB  
832 Academic Publishers, 61-77.

833 **Pons TL, Westbeek MHM. 2004.** Analysis of differences in photosynthetic nitrogen-use efficiency between  
834 four contrasting species. *Physiologia Plantarum* **122**: 68-78.

835 **Poorter H, Evans JR. 1998.** Photosynthetic nitrogen-use efficiency of species that differ inherently in specific  
836 leaf area. *Oecologia* **116**: 26-37.

837 **Quesada CA, Phillips OL, Schwarz M, Czimczik CI, Baker TR, Patino S, Fyllas NM, Hodnett MG, Herrera R,**  
838 **Almeida S et al. 2012.** Basin-wide variations in Amazon forest structure and function are mediated  
839 by both soils and climate. *Biogeosciences* **9**: 2203-2246.

840 **Raghothama KG. 1999.** Phosphate acquisition. *Annual Review of Plant Physiology and Plant Molecular*  
841 *Biology* **50**: 665-693.

842 **Randall DD, Miernyk JA, David NR, Budde RJA, Schuller KA, Fang TK, Gemel J. 1990.** Phosphorylation of the  
843 leaf mitochondrial pyruvate dehydrogenase complex and inactivation of the complex in the light.  
844 *Current Topics in Plant Biochemistry and Physiology* **9**: 313-328.

845 **Rao IM, Terry N. 1995.** Leaf phosphate status, photosynthesis, and carbon partitioning in sugar-beet. IV.  
846 Changes with time following increased supply of phosphate to low-phosphate plants. *Plant*  
847 *Physiology* **107**: 1313-1321.

848 **Reich PB, Oleksyn J. 2004.** Global patterns of plant leaf N and P in relation to temperature and latitude.  
849 *Proceedings of the National Academy of Sciences of the United States of America* **101**: 11001-11006.

850 **Reich PB, Oleksyn J, Wright IJ. 2009.** Leaf phosphorus influences the photosynthesis-nitrogen relation: a  
851 cross-biome analysis of 314 species. *Oecologia* **160**: 207-212.

852 **Reich PB, Tjoelker MG, Machado JL, Oleksyn J. 2006.** Universal scaling of respiratory metabolism, size and  
853 nitrogen in plants. *Nature* **439**: 457-461.

854 **Reich PB, Tjoelker MG, Pregitzer KS, Wright IJ, Oleksyn J, Machado JL. 2008.** Scaling of respiration to  
855 nitrogen in leaves, stems and roots of higher land plants. *Ecology Letters* **11**: 793-801.

856 **Reich PB, Walters MB. 1994.** Photosynthesis-nitrogen relations in Amazonian tree species. II. Variation in  
857 nitrogen vis-a-vis specific leaf area influences mass-based and area-based expressions. *Oecologia* **97**:  
858 73-81.

859 **Rowland L, Zaragoza-Castells J, Bloomfield KJ, Turnbull MH, Bonal D, Burban B, Salinas N, Cosio E, Metcalfe**  
860 **DJ, Ford A et al. 2016.** Scaling leaf respiration with nitrogen and phosphorus in tropical forests  
861 across two continents. *New Phytologist* **In press (doi: 10.1111/nph.13992)**.

862 **Ryan MG. 1995.** Foliar maintenance respiration of subalpine and boreal trees and shrubs in relation to  
863 nitrogen content. *Plant, Cell and Environment* **18**: 765-772.

864 **Scheiner D. 1976.** Determination of ammonia and Kjeldahl nitrogen by indophenol method. *Water Research*  
865 **10**: 31-36.

866 **Sterner RW, Elser JJ. 2002.** *Ecological Stoichiometry: The Biology of Elements from Molecules to the*  
867 *Biosphere*. Princeton, NJ: Princeton University Press.

868 **Stitt M, Schulze D. 1994.** Does Rubisco control the rate of photosynthesis and plant growth - an exercise in  
869 molecular ecophysiology. *Plant, Cell & Environment* **17**: 465-487.

870 **Tcherkez G, Bligny R, Gout E, Mahe A, Hodges M, Cornic G. 2008.** Respiratory metabolism of illuminated  
871 leaves depends on CO<sub>2</sub> and O<sub>2</sub> conditions. *Proceedings of the National Academy of Sciences, USA*  
872 **105**: 797-802.

873 **Thomas DS, Montagu KD, Conroy JP. 2006.** Leaf inorganic phosphorus as a potential indicator of phosphorus  
874 status, photosynthesis and growth of *Eucalyptus grandis* seedlings. *Forest Ecology and Management*  
875 **223**: 267-274.

876 **Turnbull MH, Griffin KL, Fyllas NM, Lloyd J, Meir P, Atkin OK. 2016.** Separating species and environmental  
877 determinants of leaf functional traits in temperate rainforest plants along a soil-development  
878 chronosequence. *Functional Plant Biology* **43**: 751-765.

879 **Warren CR, Adams MA. 2002.** Phosphorus affects growth and partitioning of nitrogen to Rubisco in *Pinus*  
880 *pinaster*. *Tree Physiology* **22**: 11-19.

881 **Warren CR, Adams MA, Chen ZL. 2000.** Is photosynthesis related to concentrations of nitrogen and rubisco  
882 in leaves of Australian native plants? *Australian Journal of Plant Physiology* **27**: 407-416.

883 **Warren CR, Dreyer E, Adams MA. 2003.** Photosynthesis-Rubisco relationships in foliage of *Pinus sylvestris* in  
884 response to nitrogen supply and the proposed role of Rubisco and amino acids as nitrogen stores.  
885 *Trees* **17**: 359-366.

886 **Warton DI, Duursma RA, Falster DS, Taskinen S. 2012.** SMART 3-an R package for estimation and inference  
887 about allometric lines. *Methods in Ecology and Evolution* **3**: 257-259.

888 **Warton DI, Wright IJ, Falster DS, Westoby M. 2006.** Bivariate line-fitting methods for allometry. *Biological*  
889 *Reviews* **81**: 259-291.

890 **Way DA, Holly C, Bruhn D, Ball MC, Atkin OK. 2015.** Diurnal and seasonal variation in light and dark  
891 respiration in field-grown *Eucalyptus pauciflora*. *Tree Physiology* **35**: 840-849.

892 **Way DA, Yamori W. 2014.** Thermal acclimation of photosynthesis: on the importance of adjusting our  
893 definitions and accounting for thermal acclimation of respiration. *Photosynthesis Research* **119**: 89-  
894 100.

895 **Weerasinghe LK, Creek D, Crous KY, Xiang S, Liddell MJ, Turnbull MH, Atkin OK. 2014.** Canopy position  
896 affects the relationships between leaf respiration and associated traits in a tropical rainforest in Far  
897 North Queensland. *Tree Physiology* **34**: 564-584.

898 **Wehr R, Munger JW, McManus JB, Nelson DD, Zahniser MS, Davidson EA, Wofsy SC, Saleska SR. 2016.**  
899 Seasonality of temperate forest photosynthesis and daytime respiration. *Nature* **534**: 680-683.

900 **Whitehead D, Boelman N, Turnbull M, Griffin K, Tissue D, Barbour M, Hunt J, Richardson S, Peltzer D. 2005.**  
901 Photosynthesis and reflectance indices for rainforest species in ecosystems undergoing progression  
902 and retrogression along a soil fertility chronosequence in New Zealand. *Oecologia* **144**: 233-244.

903 **Wohlfahrt G, Bahn M, Haslwanter A, Newesely C, Cernusca A. 2005.** Estimation of daytime ecosystem  
904 respiration to determine gross primary production of a mountain meadow. *Agricultural and Forest*  
905 *Meteorology* **130**: 13-25.

906 **Woodrow IE, Ellis JR, Jellings A, Foyer CH. 1984.** Compartmentation and fluxes of inorganic-phosphate in  
907 photosynthetic cells. *Planta* **161**: 525-530.

908 **Wright IJ, Reich PB, Westoby M. 2003.** Least-cost input mixtures of water and nitrogen for photosynthesis.  
909 *American Naturalist* **161**: 98-111.

910 **Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen**  
911 **JHC, Diemer M et al. 2004.** The worldwide leaf economics spectrum. *Nature* **428**: 821-827.

912 **Wright IJ, Westoby M. 2003.** Nutrient concentration, resorption and lifespan: leaf traits of Australian  
913 sclerophyll species. *Functional Ecology* **17**: 10-19.

914 **Xiang S, Reich PB, Sun S, Atkin OK. 2013.** Contrasting leaf trait scaling relationships in tropical and  
915 temperate wet forest species. *Functional Ecology* **27**: 522-534.

916

917 [The following Supporting Information is available for this article:](#)

918 [Notes S1 Analysing trait-trait relationships](#)

919 [Notes S2 Light inhibition of leaf respiration to photorespiratory metabolism: Hypothesis 5](#)

920 **Supplementary Figures and Tables:**

921 ~~**Table S1** Species averages  $\pm$  standard error of the mean per nutrient treatment for leaf mass per~~  
922 ~~area ratio ( $M_a$ ), area-based leaf nitrogen ( $N_a$ ) and leaf phosphorus ( $P_a$ ), light-saturated~~  
923 ~~photosynthesis ( $A_a$ ), leaf respiration in darkness ( $R_{Dark,a}$ ) and in the light ( $R_{Light,a}$ ).~~

924 ~~**Table S2** Means  $\pm$  standard errors for several leaf (nitrogen (N), phosphorus (P) and leaf mass per~~  
925 ~~area ratio,  $M_a$ ) and metabolic traits (light-saturated photosynthesis ( $A_a$ ), leaf respiration in darkness~~  
926 ~~( $R_{Dark,a}$ ) and in the light ( $R_{Light,a}$ ) on either an area- or mass-basis (indicated with a or m-subscripts~~  
927 ~~respectively) for each functional group and nutrient treatment.~~

928 ~~**Table S3** Means  $\pm$  standard error of mass-based total soluble sugars, starch and TNC (total non-~~  
929 ~~structural carbohydrates) on 16 woody species for each nutrient treatment.~~

930 ~~**Table S4** Standardized Major Axis regression slopes and their confidence intervals for mass-based~~  
931 ~~log-transformed bivariate relationships in each nutrient treatment.~~

932 ~~**Table S5** Standardized Major Axis regression slopes and their confidence intervals for area-based~~  
933 ~~log-transformed bivariate relationships in each PFT or between herbaceous and woody PFT groups.~~

934

935 **Fig. S1** Box plots showing the variation of leaf structural and chemical traits for four different  
936 nutrient treatment combination of nitrogen (N) and phosphorus (P) supply:  $H_NH_P$  (high N—high P),  
937  $H_NL_P$  (High N—Low P),  $L_NH_P$  (Low N—High P) and  $L_NL_P$  (Low N—Low P). Traits shown are: (a) leaf  
938 mass per unit leaf area ( $M_a$ ); (b) area-based leaf nitrogen concentration ( $N_a$ ); and (c) area-based leaf  
939 phosphorus concentration ( $P_a$ ).

940

941 **Fig. S2** [RLog-log](#) relationships between (a, d) mass-based light-saturated photosynthesis ( $A_a$ ), (b, e)  
942 mass-based dark respiration ( $R_{Dark,a}$ ) and (c, f) mass-based mitochondrial respiration in the light



943  $(R_{\text{Light},a})$  as a function ~~mass-based~~ leaf nitrogen concentrations  $(N_m)$  (upper panels) and as a function  
944 of leaf mass per unit leaf area  $(M_a)$  (lower panels). ~~SMA analyses were done on individual~~  
945 ~~observations within each nutrient combination treatment:  $H_N H_P$  (high N — high P, red),  $H_N L_P$  (High N~~  
946 ~~— Low P, green),  $L_N H_P$  (Low N — High P, blue) and  $L_N L_P$  (Low N — Low P, yellow).~~

947  
948 **Fig. S3** Log-log Relationships between area-based dark respiration  $(R_{\text{Dark},a})$  and light-saturated  
949 photosynthesis showing individual observations within each plant functional type across all nutrient  
950 treatments: C4G, grass with C4 photosynthetic pathway; C3G, grass species with C3 photosynthetic  
951 pathway; C3H, C3 metabolism herb; S, shrub; BLT, broad-leaved tree; NLT, needle-leaved tree. All  
952 values are shown on a  $\log_{10}$  scale. See Table S5 for standardized major axis (SMA) regression  
953 outputs.

954 ~~Log-log relationships between (a) area-based dark respiration  $(R_{\text{Dark},a})$  and light-saturated~~  
955 ~~photosynthesis and (b) area-based mitochondrial respiration in the light  $(R_{\text{Light},a})$  showing individual~~  
956 ~~observations within herbaceous (herb) and woody species. See Table 5 for standardized major axis~~  
957 ~~(SMA) regression outputs.~~

958  
959 **Fig. S4** ~~Log-log relationships between (a) N-based dark respiration  $(R_{\text{Dark},N})$  and (b) photosynthetic~~  
960 ~~nitrogen use efficiency  $(A_N)$  as a function of N:P ratio for six plant functional types.~~

961  
962 **Fig. S5** ~~Log-log relationships of nitrogen-use efficiency (via photosynthesis-N relationships)~~  
963 ~~between  $p$ Proteoid  $B$ broadleaved species (yellow) and Broadleaved trees (BIT, blue) in each~~  
964 ~~nutrient treatment:  $H_N H_P$  (high N — high P),  $H_N L_P$  (High N — Low P),  $L_N H_P$  (Low N — High P) and  $L_N L_P$~~   
965 ~~(Low N — Low P). Nitrogen-use efficiency was significantly ( $P < 0.05$ ) higher for Proteoids when~~  
966 ~~grown in low P conditions (right panels) but not when P supply was high (left panels).~~

967 **Table S1** Species averages per nutrient treatment for leaf mass per area ratio  $(M_a)$ , area-based leaf  
968 nitrogen and leaf phosphorus, light-saturated photosynthesis, leaf respiration in darkness and in  
969 the light.

970 **Table S2** Means for several leaf structural/chemical composition and metabolic traits on either an  
971 area- or mass basis.

972 [Table S3](#) Means of mass-based total soluble sugars, starch and total non-structural carbohydrates  
973 [on 16 woody species for each nutrient treatment.](#)

974 [Table S4](#) Standardized Major Axis regression slopes and their confidence intervals for mass-based  
975 [log transformed bivariate relationships in each nutrient treatment.](#)

976 [Table S5](#) Standardized Major Axis regression slopes and their confidence intervals for area-based  
977 [log transformed bivariate relationships in each PFT or between herbaceous and woody PFT groups.](#)

978 **Table 1 Two-way ANOVA testing for differences between PFT and Nutrient treatments (Treatm).**

979

Source	$M_a$		$N_m$		$P_m$		$N_a$		$P_a$		$A_a$		$R_{Dark,a}$		$R_{Light,a}$	
	DF	F	DF	F	DF	F	DF	F	DF	F	DF	F	DF	F	DF	F
PFT	5	204 <sup>***</sup>	5	42.8 <sup>***</sup>	5	43.7 <sup>***</sup>	5	142 <sup>***</sup>	5	38.8 <sup>***</sup>	5	12.4 <sup>***</sup>	5	50.7 <sup>***</sup>	5	10.8 <sup>***</sup>
Treatm	3	1.9 <sup>ns</sup>	3	83.2 <sup>***</sup>	3	253 <sup>***</sup>	3	78.9 <sup>***</sup>	3	181 <sup>***</sup>	3	7.2 <sup>***</sup>	3	8.1 <sup>***</sup>	3	3.5 <sup>*</sup>
PFT x Treatm	15	1.4 <sup>ns</sup>	15	2.4 <sup>**</sup>	15	8.3 <sup>***</sup>	15	2.38 <sup>**</sup>	15	6.3 <sup>***</sup>	15	1.1 <sup>ns</sup>	15	1.9 <sup>*</sup>	15	0.34 <sup>ns</sup>
<del>Error</del>	777		779		779		768		768		744		735		566	
<u>Within</u>	777		779		779		768		768		744		735		566	
Total	800	45.7 <sup>***</sup>	802	23.4 <sup>***</sup>	802	58.4 <sup>***</sup>	791	43.9 <sup>***</sup>	791	42.9 <sup>***</sup>	767	4.7 <sup>***</sup>	758	14.1 <sup>***</sup>	589	4.1 <sup>***</sup>

980

981 Variables shown are: leaf mass per unit area ( $M_a$ ), mass-based leaf nitrogen ( $N_m$ ) and leaf phosphorus ( $P_m$ ), area-based leaf nitrogen values ( $N_a$ )  
 982 and phosphorus ( $P_a$ ), area-based rates of light-saturated photosynthesis ( $A_a$ ) and area-based rates of leaf respiration in darkness ( $R_{Dark,a}$ ) and in  
 983 the light ( $R_{Light,a}$ ). Degrees of freedom (DF) and F-ratio (F) are indicated for each variable with statistical significance indicated as follows: \*\*\*  $P$   
 984 < 0.0001, \*\*  $P$  < 0.01, \*  $P$  < 0.05, ns is non-significant. All models were highly significant with error and total degrees of freedom varying  
 985 depending on the variable.

**Table 2 Means and standard error for leaf traits and metabolic fluxes measured on individual plants across plantPFTs-functional groups.**

Trait	Units	Treatment			
		High N High P	High N Low P	Low N High P	Low N Low P
$N_m$	$\text{mg g}^{-1}$	$26.69 \pm 0.85^a$	$22.21 \pm 0.64^b$	$16.42 \pm 0.65^c$	$15.31 \pm 0.55^c$
$P_m$	$\text{mg g}^{-1}$	$3.88 \pm 0.20^a$	$1.07 \pm 0.09^b$	$5.52 \pm 0.30^c$	$1.69 \pm 0.12^d$
$N:P$	dimensionless	$8.57 \pm 0.29^a$	$34.56 \pm 1.55^b$	$4.72 \pm 0.26^c$	$15.00 \pm 0.87^d$
$M_A$	$\text{g m}^{-2}$	$113.18 \pm 7.22^a$	$118.82 \pm 5.77^a$	$109.75 \pm 5.71^a$	$119.96 \pm 6.16^a$
$A_a$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$15.59 \pm 0.48^a$	$13.01 \pm 0.48^b$	$12.17 \pm 0.43^b$	$12.13 \pm 0.41^b$
$A_m$	$\text{nmol g}^{-1} \text{s}^{-1}$	$245.42 \pm 17.77^a$	$168.02 \pm 12.56^b$	$162.00 \pm 9.83^b$	$149.59 \pm 8.15^b$
$A_N$	$\mu\text{mol g}_N \text{s}^{-1}$	$8.65 \pm 0.41^a$	$6.92 \pm 0.39^b$	$9.56 \pm 0.36^c$	$9.55 \pm 0.36^c$
$A_P$	$\mu\text{mol g}_P \text{s}^{-1}$	$72.99 \pm 3.80^a$	$195.35 \pm 8.98^b$	$43.84 \pm 2.72^c$	$131.42 \pm 6.90^d$
$R_{\text{Dark},a}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$1.07 \pm 0.05^a$	$0.90 \pm 0.04^b$	$0.78 \pm 0.04^{bc}$	$0.74 \pm 0.03^c$
$R_{\text{Dark},m}$	$\text{nmol g}^{-1} \text{s}^{-1}$	$14.67 \pm 0.87^a$	$10.68 \pm 0.66^b$	$9.94 \pm 0.61^b$	$8.79 \pm 0.51^c$
$R_{\text{Dark},N}$	$\mu\text{mol g}_N \text{s}^{-1}$	$0.56 \pm 0.03^b$	$0.47 \pm 0.03^a$	$0.60 \pm 0.03^c$	$0.57 \pm 0.03^{bc}$
$R_{\text{Dark},P}$	$\mu\text{mol g}_P \text{s}^{-1}$	$4.87 \pm 0.30^a$	$14.24 \pm 0.87^b$	$2.50 \pm 0.15^c$	$7.62 \pm 0.45^d$
$R_{\text{Light},a}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$0.86 \pm 0.05^a$	$0.76 \pm 0.05^{ab}$	$0.67 \pm 0.05^{bc}$	$0.61 \pm 0.05^c$
$R_{\text{Light},m}$	$\text{nmol g}^{-1} \text{s}^{-1}$	$11.83 \pm 1.12^a$	$9.41 \pm 1.06^b$	$9.18 \pm 1.10^b$	$8.31 \pm 1.14^b$
$R_{\text{Light},N}$	$\mu\text{mol g}_N \text{s}^{-1}$	$0.43 \pm 0.03^a$	$0.39 \pm 0.03^a$	$0.52 \pm 0.06^a$	$0.49 \pm 0.06^a$
$R_{\text{Light},P}$	$\mu\text{mol g}_P \text{s}^{-1}$	$3.77 \pm 0.30^c$	$11.31 \pm 0.91^a$	$2.01 \pm 0.05^d$	$6.10 \pm 0.53^b$
$R_{\text{Dark}:A}$	dimensionless	$0.076 \pm 0.004^{ab}$	$0.086 \pm 0.007^a$	$0.070 \pm 0.004^{ab}$	$0.064 \pm 0.002^b$
$R_{\text{Light}:A}$		$0.056 \pm 0.004^{ab}$	$0.061 \pm 0.006^a$	$0.052 \pm 0.005^{bc}$	$0.046 \pm 0.004^c$
$R_{\text{Light}:R_{\text{Dark}}}$		$0.701 \pm 0.03^a$	$0.787 \pm 0.06^a$	$0.741 \pm 0.04^a$	$0.731 \pm 0.05^a$

The sample size for each leaf trait varied between 217-220 (171-183 for  $R_{\text{Light}}$  variables). Leaf traits presented here are mass-based nitrogen ( $N_m$ ) and phosphorus ( $P_m$ ) concentrations, their ratio ( $N:P$ ) and leaf mass per area ratio ( $M_A$ ). Metabolic traits are light saturated photosynthesis ( $A$ ) and dark respiration ( $R_{\text{Dark}}$ ), presented on an area-, mass, N- and P-basis as well as the  $R/A$  ratio. Lowercase letters indicated significant differences between different nutrient treatments at  $P < 0.05$  in a least square means Student-t post-hoc test.

995 **Table 3 Means and standard error of the mean across Plant Functional Type (PFT) for soluble sugars,**  
 996 **starch and non-structural carbohydrates (TNC) both on a mass-basis (upper panel) and an area-basis**  
 997 **(bottom panel), including sample size (n).**

Treatment	Units	H <sub>N</sub> H <sub>P</sub>	H <sub>N</sub> L <sub>P</sub>	L <sub>N</sub> H <sub>P</sub>	L <sub>N</sub> L <sub>P</sub>
n		217	218	218	220
<b>MASS-BASED</b>					
Soluble Sugars	mg g <sup>-1</sup>	51.85 ± 3.35 <sup>a</sup>	43.13 ± 3.62 <sup>ab</sup>	39.34 ± 2.76 <sup>b</sup>	39.81 ± 2.68 <sup>b</sup>
Starch	mg g <sup>-1</sup>	35.20 ± 4.57 <sup>b</sup>	56.74 ± 7.87 <sup>a</sup>	41.99 ± 7.27 <sup>b</sup>	62.41 ± 10.21 <sup>ab</sup>
TNC	mg g <sup>-1</sup>	87.05 ± 6.53 <sup>a</sup>	99.88 ± 8.84 <sup>a</sup>	81.32 ± 8.21 <sup>a</sup>	102.22 ± 10.44 <sup>a</sup>
<b>AREA-BASED</b>					
Soluble Sugars	g m <sup>-2</sup>	7.44 ± 1.07 <sup>a</sup>	6.20 ± 0.68 <sup>a</sup>	5.29 ± 0.56 <sup>a</sup>	6.20 ± 0.57 <sup>a</sup>
Starch	g m <sup>-2</sup>	3.80 ± 0.44 <sup>b</sup>	6.89 ± 1.08 <sup>a</sup>	5.12 ± 0.99 <sup>b</sup>	8.52 ± 1.46 <sup>a</sup>
TNC	g m <sup>-2</sup>	11.24 ± 1.32 <sup>bc</sup>	13.09 ± 1.26 <sup>ab</sup>	10.41 ± 1.19 <sup>c</sup>	14.72 ± 1.53 <sup>a</sup>
<i>M<sub>a</sub></i> (TNC <sub>corr</sub> )	g m <sup>-2</sup>	137.7 ± 16.2 <sup>a</sup>	130.6 ± 7.8 <sup>a</sup>	128.0 ± 11.4 <sup>a</sup>	143.8 ± 11.2 <sup>a</sup>

998

999 Different letters indicate statistical differences between treatments at  $P < 0.05$  in a Least square means  
 000 Student-t post-hoc test after log transformation. Also shown are  $M_a$  corrected for area-based TNC (TNC<sub>corr</sub>).  
 001 Nutrient treatments are defined as follows: High N–High P (H<sub>N</sub>H<sub>P</sub>), High N–Low P (H<sub>N</sub>L<sub>P</sub>), Low N–High P  
 002 (L<sub>N</sub>H<sub>P</sub>) and Low N–Low P (L<sub>N</sub>L<sub>P</sub>).

**Table 4 Standardized Major Axis coefficients for area-based log-transformed bivariate relationships for Figs 3, 4 and 5.**

Fig.	Model	Treat.	n	R <sup>2</sup>	p-value	Starting model				Simpler model, common slopes						
						Scaling exponent ( $\beta_1$ )		Proportionality		H0: no diff.		Proportionality		H0: no diff.		H0: no diff.
						( $\pm$ CI)	coefficient ( $\beta_0$ )	( $\pm$ CI)	coefficient ( $\beta_0$ )	in $\beta_1$	Pairwise	Common $\beta_1$ ( $\pm$ CI)	coefficient ( $\beta_0$ ) ( $\pm$ CI)	in $\beta_0$	Pairwise	Common $\beta_0$ ( $\pm$ CI)
				(p-value)	comp.				(p-value)	comp.		(p-value)				
3a	N <sub>a</sub> - M <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	182	0.675	< 0.0001	0.901 (0.829, 0.98)	-1.424 (-1.571, -1.277)	0.4955	0.854 (0.814, 0.897)	-1.334 (-1.417, -1.251)	< 0.0001	A	< 0.0001			
		H <sub>N</sub> L <sub>P</sub>	177	0.603	< 0.0001	0.838 (0.763, 0.921)	-1.356 (-1.514, -1.198)			-1.388 (-1.473, -1.302)		B				
		L <sub>N</sub> H <sub>P</sub>	186	0.443	< 0.0001	0.835 (0.749, 0.93)	-1.518 (-1.697, -1.339)			-1.557 (-1.642, -1.471)		C				
		L <sub>N</sub> L <sub>P</sub>	184	0.466	< 0.0001	0.822 (0.739, 0.915)	-1.513 (-1.689, -1.337)			-1.577 (-1.663, -1.49)		C				
3b	P <sub>a</sub> - M <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	182	0.475	< 0.0001	1.053 (0.947, 1.171)	-2.608 (-2.827, -2.39)	< 0.0001	A							
		H <sub>N</sub> L <sub>P</sub>	177	0.025	0.0361	0.95 (0.821, 1.101)	-3.034 (-3.316, -2.752)					A				
		L <sub>N</sub> H <sub>P</sub>	186	0.369	< 0.0001	1.463 (1.304, 1.642)	-3.288 (-3.623, -2.953)					B				
		L <sub>N</sub> L <sub>P</sub>	184	0.147	< 0.0001	1.143 (0.999, 1.308)	-3.21 (-3.52, -2.899)					A				
3c	N <sub>a</sub> - P <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	182	0.624	< 0.0001	0.856 (0.782, 0.936)	0.808 (0.756, 0.861)	< 0.0001	A							
		H <sub>N</sub> L <sub>P</sub>	177	0.057	0.0014	0.882 (0.764, 1.019)	1.321 (1.167, 1.474)					A				
		L <sub>N</sub> H <sub>P</sub>	186	0.265	< 0.0001	0.57 (0.504, 0.646)	0.358 (0.312, 0.404)					B				
		L <sub>N</sub> L <sub>P</sub>	184	0.119	< 0.0001	0.719 (0.627, 0.825)	0.795 (0.693, 0.898)					A				
4a	A <sub>a</sub> - N <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	182	0.094	< 0.0001	0.747 (0.649, 0.858)	0.926 (0.878, 0.975)	0.0008	C							
		H <sub>N</sub> L <sub>P</sub>	177	0.070	0.0004	1.135 (0.983, 1.31)	0.702 (0.633, 0.777)					A				
		L <sub>N</sub> H <sub>P</sub>	186	0.250	< 0.0001	0.921 (0.813, 1.045)	0.929 (0.894, 0.963)					B				
		L <sub>N</sub> L <sub>P</sub>	184	0.211	< 0.0001	0.895 (0.786, 1.019)	0.935 (0.899, 0.97)					B,C				
4b	A <sub>a</sub> - P <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	182	0.026	0.0306	0.639 (0.553, 0.738)	1.53 (1.462, 1.598)	< 0.0001	B							
		H <sub>N</sub> L <sub>P</sub>	177	0.049	0.0030	1.001 (0.866, 1.157)	2.2 (2.026, 2.375)					A				
		L <sub>N</sub> H <sub>P</sub>	186	0.057	0.0010	0.526 (0.457, 0.605)	1.258 (1.207, 1.309)					B				
		L <sub>N</sub> L <sub>P</sub>	184	0.017	0.0745											
4c	A <sub>a</sub> - M <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	183	0.001	0.6419											
		H <sub>N</sub> L <sub>P</sub>	177	0.003	0.4969											
		L <sub>N</sub> H <sub>P</sub>	189	0.023	0.0379	0.77 (0.668, 0.887)	-0.469 (-0.687, -0.251)	0.9540	0.767 (0.694, 0.848)	-0.464 (-0.621, -0.308)	0.4150	-0.475 (-0.629, -0.321)	0.5442			
		L <sub>N</sub> L <sub>P</sub>	190	0.020	0.0505	0.765 (0.664, 0.882)	-0.485 (-0.705, -0.264)			-0.489 (-0.648, -0.33)						
4d	R <sub>dark,a</sub> - N <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	182	0.140	< 0.0001	1.03 (0.899, 1.18)	-0.363 (-0.427, -0.299)	0.2024	1.142 (1.068, 1.221)	-0.397 (-0.453, -0.341)	< 0.0001	B	< 0.0001			
		H <sub>N</sub> L <sub>P</sub>	177	0.080	0.0001	1.271 (1.102, 1.466)	-0.508 (-0.585, -0.432)			-0.469 (-0.524, -0.414)		C				
		L <sub>N</sub> H <sub>P</sub>	186	0.223	< 0.0001	1.121 (0.987, 1.274)	-0.296 (-0.34, -0.253)			-0.299 (-0.34, -0.257)		A				
		L <sub>N</sub> L <sub>P</sub>	184	0.188	< 0.0001	1.171 (1.027, 1.336)	-0.347 (-0.394, -0.299)			-0.343 (-0.388, -0.299)		A,B				
4e	R <sub>dark,a</sub> - P <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	182	0.049	0.0027	0.881 (0.764, 1.017)	0.47 (0.378, 0.561)	< 0.0001	B							
		H <sub>N</sub> L <sub>P</sub>	177	0.035	0.0127	1.121 (0.969, 1.298)	1.17 (0.973, 1.367)					A				
		L <sub>N</sub> H <sub>P</sub>	186	0.208	< 0.0001	0.64 (0.562, 0.728)	0.105 (0.051, 0.159)					C				
		L <sub>N</sub> L <sub>P</sub>	184	0.054	0.0015	0.842 (0.731, 0.971)	0.585 (0.46, 0.71)					B				
4f	R <sub>dark,a</sub> - M <sub>a</sub>	H <sub>N</sub> H <sub>P</sub>	183	0.074	0.0002	0.929 (0.807, 1.069)	-1.834 (-2.091, -1.577)	0.5230	0.969 (0.903, 1.04)	-1.912 (-2.053, -1.77)	< 0.0001	A	0.0563			
		H <sub>N</sub> L <sub>P</sub>	177	0.045	0.0047	1.066 (0.922, 1.232)	-2.232 (-2.544, -1.919)			-2.041 (-2.186, -1.895)		B				
		L <sub>N</sub> H <sub>P</sub>	189	0.081	< 0.0001	0.938 (0.817, 1.076)	-2.002 (-2.259, -1.744)			-2.063 (-2.205, -1.922)		B				
		L <sub>N</sub> L <sub>P</sub>	190	0.047	0.0026	0.958 (0.833, 1.102)	-2.116 (-2.388, -1.844)			-2.137 (-2.283, -1.992)		C				

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Table 4 contd.

Fig.	Model	Treat.	n	R <sup>2</sup>	p-value	Starting model				Simpler model, common slopes															
						Scaling exponent ( $\beta_1$ )		Proportionality		in $\beta_1$		Proportionality		in $\beta_0$		Pairwise									
						HO: no diff.				HO: no diff.															
						( $\pm$ CI)		coefficient ( $\beta_0$ ) ( $\pm$ CI)		(p-value)		comp.		Common $\beta_1$ ( $\pm$ CI)		coefficient ( $\beta_0$ ) ( $\pm$ CI)		(p-value)		comp.		Common $\beta_0$ ( $\pm$ CI)		(p-value)	
4g	$R_{light,a} - N_a$	H <sub>N</sub> H <sub>P</sub>	152	0.082	0.0004	1.299 (1.114, 1.515)		-0.629 (-0.726, -0.531)		0.0471		A													
		H <sub>N</sub> L <sub>P</sub>	138	0.041	0.0176	1.572 (1.332, 1.854)		-0.757 (-0.876, -0.639)				A,B													
		L <sub>N</sub> H <sub>P</sub>	148	0.044	0.0107	1.738 (1.482, 2.038)		-0.586 (-0.68, -0.492)				B													
		L <sub>N</sub> L <sub>P</sub>	134	0.040	0.0209	1.679 (1.42, 1.986)		-0.588 (-0.685, -0.492)				B													
4h	$R_{light,a} - P_a$	H <sub>N</sub> H <sub>P</sub>	152	0.039	0.0150	1.082 (0.925, 1.267)		0.425 (0.3, 0.55)		0.0016		B,C													
		H <sub>N</sub> L <sub>P</sub>	138	0.053	0.0068	1.455 (1.235, 1.715)		1.399 (1.115, 1.683)				A													
		L <sub>N</sub> H <sub>P</sub>	148	0.109	< 0.0001	0.969 (0.831, 1.131)		0.06 (-0.04, 0.16)				C													
		L <sub>N</sub> L <sub>P</sub>	134	0.032	0.0397	1.317 (1.113, 1.559)		0.891 (0.658, 1.125)				A,B													
4i	$R_{light,a} - M_a$	H <sub>N</sub> H <sub>P</sub>	152	0.024	0.0573									1.35 (1.244, 1.464)								-2.938 (-3.158, -2.718)			
		H <sub>N</sub> L <sub>P</sub>	138	0.019	0.1077																				
		L <sub>N</sub> H <sub>P</sub>	151	0.003	0.4968																				
		L <sub>N</sub> L <sub>P</sub>	138	0.000	0.8346																				
5a	$R_{dark,a} - A_a$	HNHP	187	0.128	< 0.0001	1.373 (1.2, 1.572)		-1.635 (-1.854, -1.416)		0.1637				1.234 (1.163, 1.311)		-1.476 (-1.573, -1.379)		0.0600				-1.45 (-1.532, -1.368)		< 0.0001	
		HNL <sub>P</sub>	187	0.191	< 0.0001	1.109 (0.974, 1.263)		-1.284 (-1.441, -1.126)								-1.415 (-1.506, -1.324)									
		LNHP	191	0.387	< 0.0001	1.222 (1.093, 1.368)		-1.435 (-1.581, -1.29)								-1.448 (-1.532, -1.364)									
		LNL <sub>P</sub>	190	0.428	< 0.0001	1.253 (1.124, 1.397)		-1.509 (-1.654, -1.364)								-1.49 (-1.574, -1.406)									
5b	$R_{light,a} - A_a$	HNHP	154	0.204	< 0.0001	1.75 (1.517, 2.017)		-2.228 (-2.524, -1.931)		0.0035		B,C													
		HNL <sub>P</sub>	145	0.094	0.0002	1.504 (1.286, 1.759)		-1.89 (-2.157, -1.623)				C													
		LNHP	151	0.190	< 0.0001	2.186 (1.89, 2.527)		-2.712 (-3.064, -2.359)				A													
		LNL <sub>P</sub>	138	0.191	< 0.0001	2.018 (1.734, 2.35)		-2.553 (-2.892, -2.214)				A,B													
5c	$R_{light,a} - R_{dark,a}$	HNHP	154	0.525	< 0.0001	1.615 (1.447, 1.804)		-0.239 (-0.284, -0.195)		0.4906				1.613 (1.518, 1.714)		-0.239 (-0.284, -0.195)		0.0948				-0.188 (-0.216, -0.161)		< 0.0001	
		HNL <sub>P</sub>	145	0.402	< 0.0001	1.536 (1.352, 1.744)		-0.181 (-0.236, -0.125)								-0.177 (-0.233, -0.121)									
		LNHP	151	0.425	< 0.0001	1.742 (1.541, 1.969)		-0.155 (-0.217, -0.093)								-0.169 (-0.225, -0.113)									
		LNL <sub>P</sub>	138	0.412	< 0.0001	1.554 (1.365, 1.769)		-0.165 (-0.231, -0.099)								-0.157 (-0.219, -0.094)									
5d	$R_{dark,a} - A_a$	Herb	361	0.165	< 0.0001	1.122 (1.021, 1.234)		-1.408 (-1.521, -1.295)		0.4462				1.864 (1.663, 2.089)		-2.359 (-2.592, -2.127)		< 0.0001				< 0.0001			
		Woody	394	0.354	< 0.0001	1.177 (1.087, 1.275)		-1.334 (-1.44, -1.227)								-2.253 (-2.459, -2.047)									
		Herb	-	-	-	1.154 (1.086, 1.227)		-1.441 (-1.519, -1.363)																	
		Woody	-	-	-	1.154 (1.086, 1.227)		-1.308 (-1.39, -1.227)																	
5e	$R_{light,a} - A_a$	Herb	267	0.104	< 0.0001	1.864 (1.663, 2.089)		-2.359 (-2.592, -2.127)		0.5959				1.9 (1.715, 2.105)		-0.059 (-0.119, 0.001)		0.5008				-2.318 (-2.472, -2.165)		< 0.0001	
		Woody	321	0.198	< 0.0001	1.789 (1.621, 1.975)		-2.253 (-2.459, -2.047)								-0.248 (-0.277, -0.219)									
5f	$R_{light,a} - R_{dark,a}$	Herb	267	0.281	< 0.0001	1.9 (1.715, 2.105)		-0.059 (-0.119, 0.001)		0.0005															
		Woody	321	0.576	< 0.0001	1.521 (1.416, 1.634)		-0.248 (-0.277, -0.219)																	

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Coefficients of determination (R<sup>2</sup>) and significance values (P-value) of each log-log trait-trait relationship are shown in addition to 95% confidence intervals (CI) of SMA slopes (scaling-exponent) and elevation (proportionality-coefficient). When scaling-exponents were significantly different between nutrient treatments ( $P < 0.05$ ) in the starting model, a post hoc test showed pairwise comparisons between treatments, indicated with capital letters. When scaling-exponents were not significantly different, SMA tests for proportionality-coefficient differences were conducted using a common scaling-exponent (simpler model). Where there was a significant difference in proportionality-coefficient of the common scaling-exponent SMA regressions, values for the proportionality-coefficient are pairwise comparisons were indicated via different letters in post-hoc testing. When elevation using a common scaling-exponent showed no significant differences, a test investigating potential shifts along a relationship was conducted (last column) indicated via a P-value.

## Figure Legends<sup>[OA5]</sup><sup>[OA6]</sup>

**Fig. 1** Theoretical log-log relationships linking metabolic rates to leaf nitrogen concentration among plant functional types. Changes associated with Hypothesis 2 and 3 are illustrated and show expected changes in slopes (scaling exponent) and/or y-axis intercepts elevation (proportionality coefficient) of log-log relationships. Ellipses represent anticipated metabolic rate-N concentration combinations in each of the four treatments (high N-high P, high N-low P, low N-high P and low N-low P) used in our study, with their standardized major axes (SMAs) shown. Note that as a consequence of Hypothesis 1, we anticipate low P supply to reduce rates of metabolism when N is abundant, but with less so when N is limiting. When N is limiting, average rates of metabolism are predicted to be slightly lower similar in high and the low P-grown plants, resulting in but with a shallower slope lower scaling proportionality-coefficient exponent in the low P-grown plants of the SMA regressions compared to high-P grown plants. Irrespective of P supply, we anticipate low N supply to result in an increase in the y-axis intercepts elevation of log-log relationships linking lower higher metabolic rates to for a given level of leaf N, reflecting increased relative allocation of leaf N to photosynthesis when N is limiting.

**Fig. 2** Box plots showing the variation of leaf structural and chemical traits and leaf metabolism of carbon fluxes for six different plant functional type (PFT) classifications across all nutrient treatments. Traits shown are: (a) mass-based leaf nitrogen concentration ( $N_m$ ); (b) mass-based leaf phosphorus concentration ( $P_m$ ); (c) leaf mass per unit leaf area ( $M_a$ ); ~~(b) area-based leaf nitrogen concentration ( $N_a$ ); and (c) area-based leaf phosphorus concentration ( $P_a$ );~~ (d) area-based light-saturated photosynthesis ( $A_a$ ); (e) area-based leaf mitochondrial respiration in darkness ( $R_{Dark,a}$ ); and (f) area-based leaf mitochondrial respiration in the absence of photorespiration ( $R_{Light,a}$ ). Data shown are for individual observations to give an indication of underlying data distribution. The boxes indicate the interquartile range and median. Observations outside the whiskers are shown as individual points. PFT classifications: C4G, grass species with C4 photosynthetic pathway; C3G, grass species with C3 photosynthetic pathway; C3H, C3 metabolism herb; S, shrub; ~~BIF~~BLT, broad-leaved tree; ~~NIT~~NLT, needle-leaved tree. Post-hoc differences among PFT are shown in Table S2 (across treatments column).



1044 **Fig. 3** Box plots showing the variation of leaf metabolism of carbon fluxes for six different plant  
1045 functional type (PFT) classifications. Traits shown are: (a) area based light saturated photosynthesis  
1046 ( $A_a$ ); (b) area based leaf mitochondrial respiration in darkness ( $R_{Dark,a}$ ); and (c) area based leaf  
1047 mitochondrial respiration in the absence of photorespiration ( $R_{Light,a}$ ). Data shown are for individual  
1048 observations to give an indication of underlying data distribution. PFT classifications: C4G, grass  
1049 with C4 photosynthetic pathway; C3G, grass species with C3 photosynthetic pathway; C3H, C3  
1050 metabolism herb; S, shrub; BITBLT, broad leaved tree; NITNLT, needle leaved tree.

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1052 **Fig. 4-3** Relationships between area-based leaf nitrogen levels ( $N_a$ ) and area-based leaf  
1053 phosphorus concentration ( $P_a$ ) concentration as a function of area-based leaf mass per unit leaf  
1054 area ( $M_a$ ) (panels (a) and (b) respectively) and the relationship between  $N_a$  versus  $P_a$  (panel (c)). All  
1055 values are shown on a  $\log_{10}$  scale. SMA analyses were done on individual observations within each  
1056 nutrient combination treatment:  $H_NH_P$  (high N – high P, red/black),  $H_NL_P$  (High N – Low P, green),  
1057  $L_NH_P$  (Low N – High P, blue) and  $L_NL_P$  (Low N – Low P, yellow/orange). See Table 4 for standardized  
1058 major axis (SMA) regression outputs.

1059  
1060 **Fig. 5-4** Relationships between: (1) area-based rates of leaf  $CO_2$  exchange light-saturated  
1061 photosynthesis ( $A_a$ ) as a function of (a, d and g) area-based leaf nitrogen concentration ( $N_a$ ), (b, e,  
1062 and h) area-based leaf phosphorus concentration ( $P_a$ ), and (c, f and i) leaf mass per unit leaf area  
1063 ( $M_a$ ). Upper, middle and lower panels show light-saturated photosynthesis ( $A_a$ ), area-based dark  
1064 respiration ( $R_{Dark,a}$ ) and mitochondrial respiration in the light ( $R_{Light,a}$ ), respectively. – All values are  
1065 shown on a  $\log_{10}$  scale. SMA analyses were done on individual observations within each nutrient  
1066 combination treatment:  $H_NH_P$  (high N – high P, red/black),  $H_NL_P$  (High N – Low P, green),  $L_NH_P$  (Low N  
1067 – High P, blue) and  $L_NL_P$  (Low N – Low P, yellow/orange). See Table 4 for standardized major axis  
1068 (SMA) regression outputs.

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1070 **Fig. 6-5** Relationships between area-based dark respiration ( $R_{Dark,a}$ ) (upper panels) as a function of  
1071 (a) area-based leaf nitrogen concentration ( $N_a$ ), (b) area-based leaf phosphorus concentration ( $P_a$ )  
1072 and (c) leaf mass per unit leaf area ( $M_a$ ), while lower panels show mitochondrial respiration in the  
1073 light ( $R_{Light,a}$ ) as a function of (d) area-based leaf nitrogen concentration ( $N_a$ ), (e) area-based leaf  
1074 phosphorus concentration ( $P_a$ ) and (f) leaf mass per unit leaf area ( $M_a$ ). All values are shown on a

log<sub>10</sub> scale. SMA analyses were done on individual observations within each nutrient combination treatment: H<sub>N</sub>H<sub>P</sub> (high N – high P, red/black), H<sub>N</sub>L<sub>P</sub> (High N – Low P, green), L<sub>N</sub>H<sub>P</sub> (Low N – High P, blue) and L<sub>N</sub>L<sub>P</sub> (Low N – Low P, yellow/orange). See Table 4 for standardized major axis (SMA) regression outputs. Regression lines are not shown when relationships were not significant.

**Fig. 7–65** Log-log trait-trait relationships on a log<sub>10</sub> scale among nutrient treatments (upper panels) between: (a) area-based dark respiration ( $R_{\text{Dark},a}$ ) and light-saturated photosynthesis ( $A_a$ ) across nutrient treatments, with the single regression line showing the overall relationship, given the lack of slope differences among nutrient treatments (Table 4)<sup>[OA7]</sup>; and (b) area-based mitochondrial respiration in the light ( $R_{\text{Light},a}$ ) and  $A_a$ ; and (c) area-based  $R_{\text{Light}}$  and area-based  $R_{\text{Dark}}$  showing SMA regression lines for each treatment. Values shown are for individual observations within each nutrient combination treatment: H<sub>N</sub>H<sub>P</sub> (high N – high P, black), H<sub>N</sub>L<sub>P</sub> (High N – Low P, green), L<sub>N</sub>H<sub>P</sub> (Low N – High P, blue) and L<sub>N</sub>L<sub>P</sub> (Low N – Low P, orange). See Table 4 for standardized major axis (SMA) regression outputs. All values are shown on a log<sub>10</sub> scale. The bottom panels (d, e, f) reflect the same relationships for individual observations within herbaceous (herb, blue) and woody species (orange) with SMA outputs in Table S5. In (a) and (b), values shown are for individual observations within each nutrient combination treatment: H<sub>N</sub>H<sub>P</sub> (high N – high P, red), H<sub>N</sub>L<sub>P</sub> (High N – Low P, green), L<sub>N</sub>H<sub>P</sub> (Low N – High P, blue) and L<sub>N</sub>L<sub>P</sub> (Low N – Low P, yellow). See Table 4 for standardized major axis (SMA) regression outputs.

**Fig. 8** Relationships between (a) area-based dark respiration ( $R_{\text{Dark},a}$ ) and light-saturated photosynthesis showing individual observations within each plant functional type across all nutrient treatments: C4G, grass with C4 photosynthetic pathway; C3G, grass species with C3 photosynthetic pathway; C3H, C3 metabolism herb; S, shrub; BITBLT, broad-leaved tree; NITNLT, needle-leaved tree. All values are shown on a log<sub>10</sub> scale. See Table 5 for standardized major axis (SMA) regression outputs.

**Fig. 9** Relationships between area-based mitochondrial respiration in the light ( $R_{\text{Light},a}$ ) and area-based dark respiration ( $R_{\text{Dark},a}$ ) showing individual observations within (a) each nutrient treatment being H<sub>N</sub>H<sub>P</sub> (high N – high P, red), H<sub>N</sub>L<sub>P</sub> (High N – Low P, green), L<sub>N</sub>H<sub>P</sub> (Low N – High P, blue) and L<sub>N</sub>L<sub>P</sub> (Low N – Low P, yellow) and (b) within woody and herbaceous plant species. All values are shown

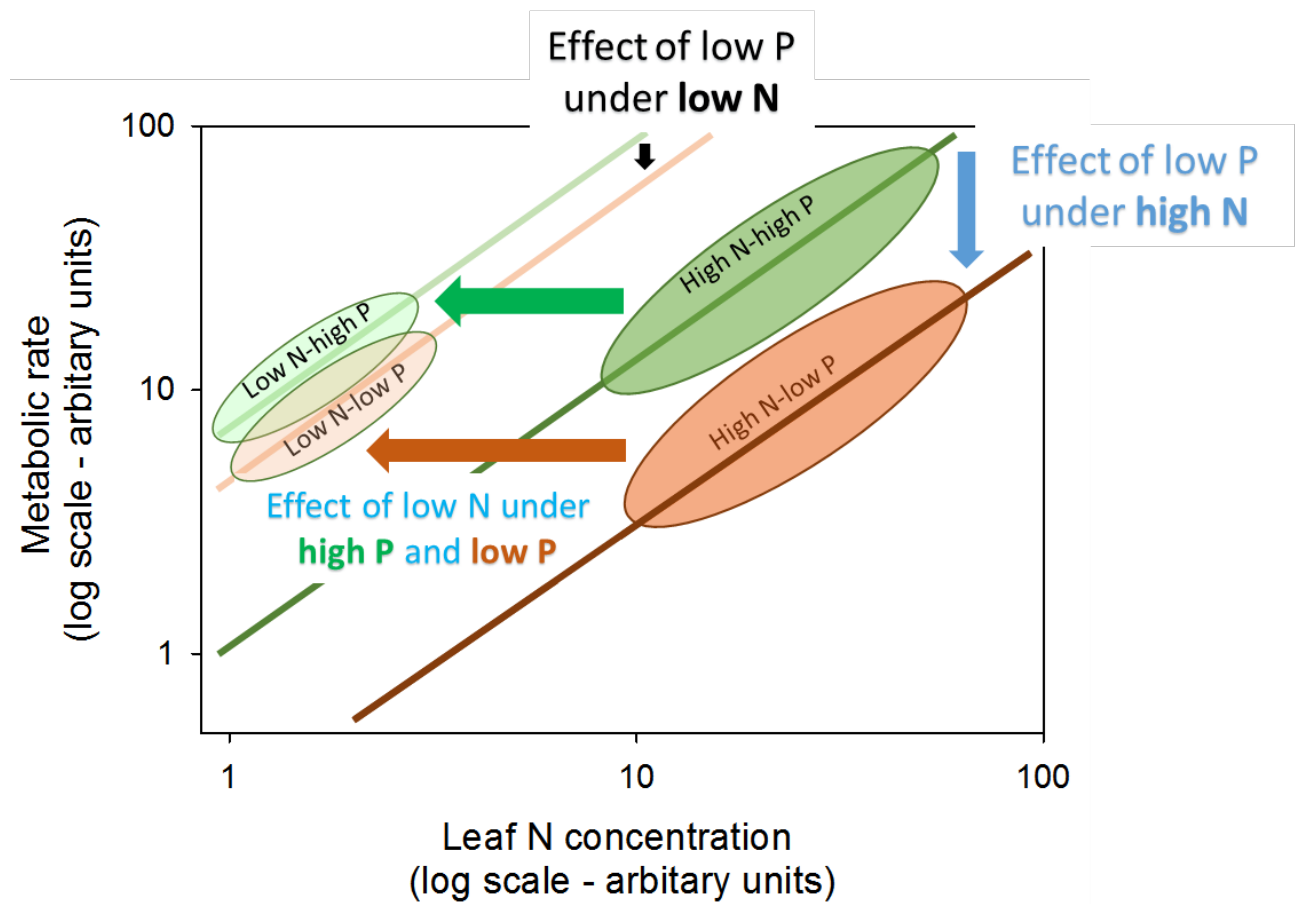
1106 ~~on a  $\log_{10}$  scale. See Table 4 for standardized major axis (SMA) regression outputs for each nutrient~~  
1107 ~~treatment and Table 5 for standardized major axis (SMA) regression outputs for woody versus~~  
1108 ~~herbaceous species~~

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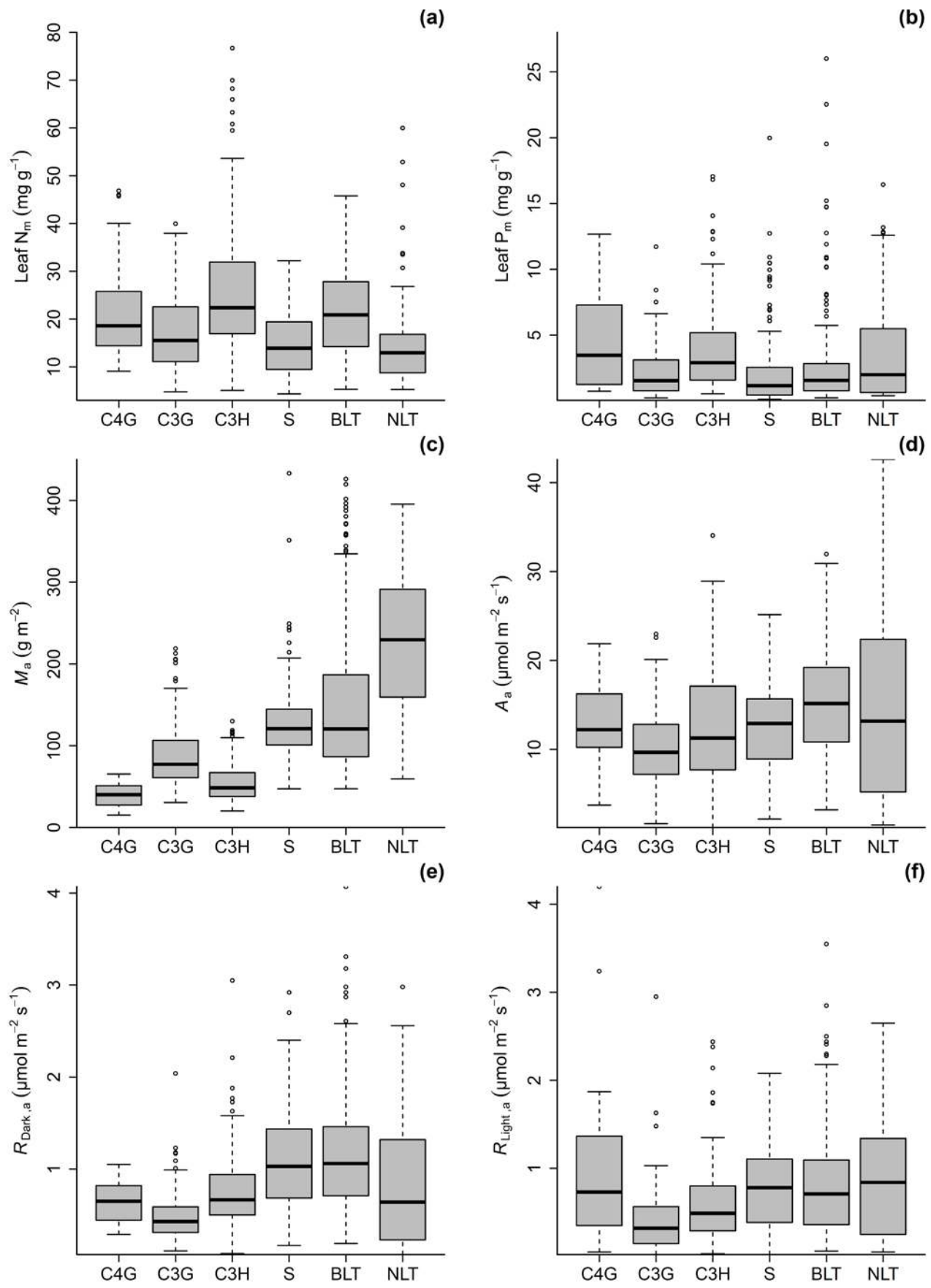
Figure 1

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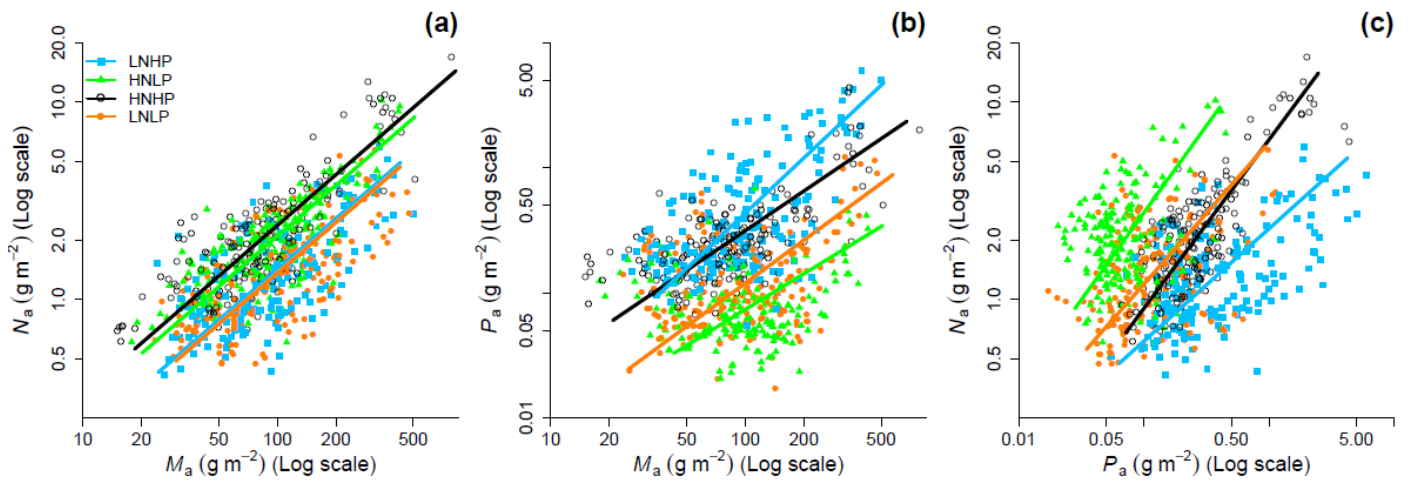
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117 Figure 3

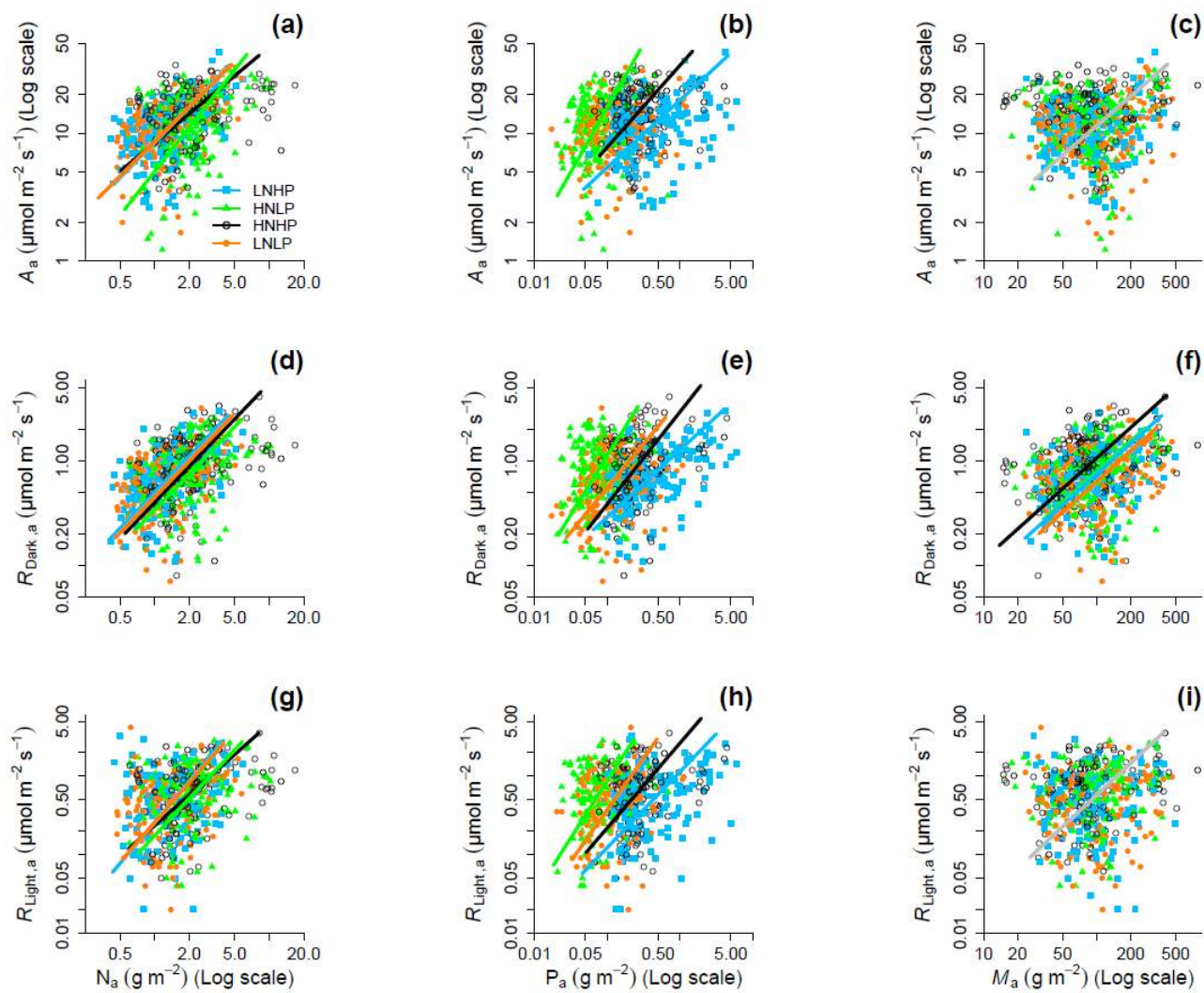
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20 Figure 4

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