Separating species and environmental determinants of leaf functional traits in temperate rainforest plants along a soil-development chronosequence

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Matthew H. Turnbull[1] (⊠), Kevin L. Griffin[2], Nikolaos M. Fyllas[3], Jon Lloyd[4,5], Patrick Meir[6,7] and Owen K. Atkin[6]

[1]Centre for Integrative Ecology, School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand
Email: matthew.turnbull@canterbury.ac.nz

[2]Department of Earth and Environmental Sciences, Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964-8000, USA

[3]Department of Ecology and Systematics, Faculty of Biology, National and Kapodistrian University of Athens, Athens 15784, Greece

[4]Department of Life Sciences, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot, Berkshire SL5 7PY, UK

[5]School of Marine and Tropical Biology, James Cook University, Cairns, 4870, Queensland, Australia

[6]Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia

[7]School of Geosciences, University of Edinburgh, Edinburgh EH8 9XP, UK

Abstract

We measured a diverse range of foliar characteristics in shrub and tree species in temperate rainforest communities along a soil chronosequence (six sites from 8 to 120,000 years) and used multilevel model analysis to attribute the proportion of variance for each trait into genetic (G, here meaning species-level), environmental (E) and residual error components. We hypothesised that differences in leaf traits would be driven primarily by changes in soil nutrient availability during ecosystem progression and retrogression. A number of leaf structural, chemical and gas exchange traits were more strongly driven by G than E effects. For leaf mass per unit area (M_A), foliar [N], net CO_2 assimilation and dark respiration rates and foliar carbohydrate concentration, the G component accounted for 60-87% of the total variance, with the variability associated with plot, the E effect, much less important. Other traits, such as foliar [P] and N:P, displayed strong E and residual effects. Analyses revealed significant reductions in the slopes of G-only bivariate relationships when compared with raw relationships, indicating that a large proportion of trait-trait relationships is species based, and not a response to environment *per se*. This should be accounted for when assessing the mechanistic basis for using such relationships in order to make predictions of responses of plants to short-term environmental change.

Key words: genotypic, phenotypic, dark respiration, soil nutrient availability, photosynthesis, temperate rainforest, phosphorus, nitrogen, carbohydrates

Introduction

An important question challenging biologists is the extent to which traits reflect evolutionary history (manifested as genotypic/taxonomic variation - G) and/or phenotypic responses to the environment (E) (Strand and Weisner 2004). This question has implications for agriculture and forestry (Hawkins *et al.* 2010) and evolutionary ecology (Miner *et al.* 2005), especially in cases where plastic responses can be shown to be adaptive. Evolutionary ecologists consider that trait plasticity is under genetic control, not independent of it (Schlichting, 1986). Clearly, phenotypic variation has genetic, environmental and residual error components (DeWitt and Scheiner 2004). This is of significance and societal concern as we attempt to predict the likely responses of ecological processes to global environmental change (Nicotra *et al.* 2010). However, our current knowledge of phenotypic plasticity is still too limited to allow for credible mechanistic predictions of future responses (Valladares *et al.* 2007; Messier *et al.* 2010; Donovan *et al.* 2014).

In order to interpret field results of plant trait responses at larger scales and apply this knowledge, the underlying mechanisms influencing the environmental and biological drivers of variation in leaf traits must be understood. We now have a better, albeit not complete, understanding of the extent to which such variation is driven by genetics or environment, particularly for leaf structural and chemical traits. The concept of a 'leaf economics spectrum' proposed by Wright et al. (2004) states that variations in some traits (e.g. foliar nitrogen concentration) are matched by variations in other related traits (e.g. mass/area relationships and metabolic rates), although the extent to which scatter in the scaling relationships is the result of genetic and/or environmental responses is often unclear. Some argue that G is more important than E (Wright *et al.* 2004), largely because many analyses are based on global patterns in light, water and temperature. However, other work (Poorter *et al.* 2009; Auger and Shipley 2013) shows that traits [such as leaf mass per unit area (M_A)] may be highly plastic along shorter gradients in response to soil N, water, and light. Therefore, there is significant potential for much of the scatter in log-log relationships to be explained by environment-dependent changes in phenotype (E).

One approach that is used to probe plant responses to environment is to investigate changes along well-defined environmental gradients. Such gradients can be seen as a "window to the

84 future" in helping us predict likely responses to environmental change, but it is essential that
85 the role of species differences (and hence evolutionary history) is explicitly recognised where
86 environmental gradients also encompass community change or successional processes. The
87 role of soil phosphorus (P) and nitrogen (N) availability is of particular interest, given their
88 potential roles in placing species along the leaf traits spectrum (Townsend *et al.* 2007; Kattge
89 *et al.* 2009; Kattge *et al.* 2011). Variations in the availability of P and N are known to play a
90 crucial role in regulating rates of plant growth and metabolism (Paul and Stitt 1993; Meir *et al.*
91 2001; de Groot *et al.* 2003; Niklas 2006; Domingues *et al.* 2010; Reich *et al.* 2010). Rates of
92 light-saturated photosynthesis (A_{sat}) are strongly determined by leaf N concentration (Evans
93 1989). Similarly, respiratory metabolism (R_D) and N metabolism are tightly linked. Thus, tissue
94 nitrogen concentration ([N]) has been observed to scale with A_{sat} (Field and Mooney 1986) and
95 R_D (Ryan 1995; Reich *et al.* 1998; Atkin *et al.* 2015). This observation has led to the proposal
96 of a 'universal' scaling relationship between [N] leaf mass per unit area (M_A), A_{sat} and R_D
97 (Wright *et al.* 2004; Reich *et al.* 2008). Deficiencies in P may also limit A_{sat} (Hidaka and
98 Kitayama 2009) and R_D (Theodorou *et al.* 1991; Gonzalez-Meler *et al.* 2001; Plaxton and
99 Podesta 2006). However, the role of P limitation, while it is likely to alter the scaling of leaf
100 physiological traits with N, is not well understood (Gleason *et al.* 2009), and has not been
101 properly tested when P and N limitations are operating concurrently.
102
103 Soil chronosequences provide opportunities for understanding how long-term nutrient
104 limitation resulting from soil development may influence patterns of ecosystem development
105 and function (Wardle 2002; Richardson *et al.* 2004). Soil development drives changes in the
106 composition, structure and functioning of ecosystems (Wardle, 2002). Availability of both N
107 and P vary during soil development as P is lost through leaching or made less available through
108 conversion to unavailable forms, and N accumulates through fixation (Walker and Syers 1976;
109 Crews *et al.* 1995). Changes in soil nutrient availability drive changes in nutrient conservation
110 through plant trait shifts (Aerts and Chapin 2000) and changes in species composition (Walker
111 and del Moral 2003). At the leaf level, responses to low nutrient availability include longer leaf
112 lifespans (Wright and Westoby 2003), higher leaf mass per unit area (Wright *et al.* 2002),
113 greater resorption of nutrients from leaves before abscission (Escudero *et al.* 1992; Richardson
114 *et al.* 2005; Hayes *et al.* 2014) and lower nutrient concentrations in mature leaves (Wright and
115 Westoby 2003). Chronosequences have an advantage over multi-biome analyses in that they
116 are geographically constrained and therefore encompass relatively small changes in
117 confounding environmental variables such as temperature and rainfall.

In this study, we re-analyse previously collected data (Atkin *et al.* 2013) to examine the relative impacts of G and E on a comprehensive range of foliar traits in six distinct communities along a temperate rainforest soil-development sequence in south-western New Zealand (the Franz Josef Chronosequence). The long-term (8-120,000 years; Richardson et al. (2004)) glacial soil-age chronosequence provides a successional/retrogressional spectrum of forest communities with both species diversity between sites and species common to more than one site. The major environmental drivers included both shifts in the relative and absolute availability of soil N and P (Richardson *et al.* 2004). Multilevel model analysis (Watanabe *et al.* 2007; Fyllas *et al.* 2009; Asner *et al.* 2014) allowed us to attribute the proportion of total variance for each foliar property into genetic, environmental (e.g. soil fertility, air temperature, precipitation) and residual error components. Using this analysis of traits in tropical rainforest tree species, Fyllas et al. (2009) found support for the role of genetic differences in determining foliar traits, but also strong environmental effects on several traits. In the present paper, we extend this statistical approach and assess a more comprehensive spectrum of leaf functional traits than is considered in trait studies. Based on the previous investigations (Turnbull *et al.* 2005; Whitehead *et al.* 2005), we hypothesised that (1) G would play a major role in leaf phenotypes, but that the large range in nutrient availability would result in the retention of a significant role for E. In addition, this approach allowed us to further examine the extent to which the slope and intercept of log-log scaling relationships amongst leaf traits can be broadly applied across landscapes. Based on hypothesis (1), we further hypothesised that (2) raw relationships between leaf traits would differ significantly from those in which the E component had been removed.

Materials and Methods

Study sites and species

Glacial activity on the western coastal strip of the South Island, New Zealand (latitude 43.2 ºS and longitude 170.3 ºE), has created a series of outwash surfaces of varying age (approximately 120,000 years to present). This study is based on the Franz Josef chronosequence, originally described by Stevens (1968). Community structure and changes in soil nutrient availability along the sequence are described in detail by Richardson *et al.* (2004). This paper describes sampling from 6 of the 9 sites described by Richardson *et al.* (2004). The sites ranged over a

151 distance of 20 km, with elevation between 140 and 240 m. In spite of compositional shifts and
152 structural changes with soil age, there were a number of species that occurred on multiple sites
153 along the chronosequence (for further description of soil and environmental conditions, see
154 Richardson *et al.* 2004). In order to increase the range of leaf functional traits measured, we
155 confined our sampling to the 3-6 most abundant species at each site. In total, our investigation
156 included 13 species from 12 different families (Table S1). Leaf traits were collected in summer
157 (2009) and have previously been presented (Atkin *et al.* 2013), but in the present study are
158 subjected to the new analysis described below. For clarity we briefly present a description of
159 the methods used.
160
161 ## Leaf structural traits and chemical composition
162 Leaves used for gas exchange measurements were weighed for fresh mass, photographed [to
163 enable subsequent calculation of leaf area using *Image J* software (http://rsbweb.nih.gov/ij/)]
164 and then oven dried at 70°C to constant mass. Subsequently, leaf samples were ground in a ball
165 mill and analysed for tissue N and P using a Technicon Auto-analyzer II (Bran + Luebbe Pty.
166 Ltd, Norderstedt Germany) and Kjeldahl acid digests (Ayub *et al.* 2011). Ground leaf material
167 was also used to analyse soluble sugars (glucose, fructose and sucrose) and starch as described
168 previously (Loveys *et al.* 2003). The mass and area data were used to determine ratios of leaf
169 dry mass to leaf area (M_A) and the inverse (i.e. specific leaf area, $1/M_A$) to allow leaf trait values
170 to be presented on both an area and mass basis and to account for recent debate on this topic
171 (Poorter *et al.* 2013; Westoby *et al.* 2013; Lloyd *et al.* 2103).
172
173 ## Gas exchange measurements
174 Measurements of foliar photosynthetic and dark respiration rates were made on fully expanded
175 leaves from the upper (sunlit) part of the canopies of each experimental tree. At each site, large,
176 woody shoots (0.3 – 0.5 m in length) were collected from 5 individuals of the three dominant
177 species in the canopy using a pruning pole or shotgun. Following collection, shoots were re-
178 cut underwater, placed in plastic bags and returned promptly to the lab (within 1 hour). Previous
179 studies have shown no differences in respiration between *in situ* leaves and leaves from
180 detached branches in a range of species, including those described here (Mitchell *et al.* 1999;
181 Turnbull *et al.* 2003; Turnbull *et al.* 2005) and we have successfully used this approach to
182 compare photosynthetic parameters between species and sites (Whitehead *et al.* 2005). Light-
183 saturated photosynthesis (A_{sat}) was determined using gas analysis systems (Li-Cor model 6400,
184 Lincoln, Nebraska, USA) equipped with CO_2 control modules at an external CO_2 concentration

(C_a) of 400 ppm, using an established protocol (Turnbull *et al.*, 2005). Leaf temperatures were maintained at 20 °C using thermoelectric coolers and a constant photon flux density (PFD) of 1500 μmol m^{-2} s^{-1} was provided by blue-red light emitting diodes mounted above the leaf cuvette. Respiration measurements were made on shoots that had been kept in the dark for 1-2 hours prior to measurement. Measurements were made at 20 ºC and an external C_a of 400 ppm. Each respiration measurement was the average of 5 individual measurements made over a 60 second period after stability was reached. After gas exchange measurements, the leaf samples were removed and photographed using a digital camera for determination of surface area. For comparative purposes, all estimates of photosynthesis and respiration in this paper are presented on a hemi-surface area basis.

Statistical analysis

The analysis we adopted here separates genetic/taxonomic (G, species-level) and plot-environmental (E) components of trait variation, as estimated from a multilevel model described in detail by Fyllas *et al.* (2009), and subsequently successfully tested for analysis of phylogenetic partitioning of foliar chemical traits (Asner and Martin 2011; Asner *et al.* 2014). Preliminary tests included: analysis of normality (Shapiro-Wilk), and homogeneity of variances (Fligner-Killeen) for each foliar property. Where properties were not normally distributed they were log10-transformed prior to analyses. One-way analysis of variance (ANOVA) was used to explore for differences between plots. All analyses were performed with the R statistical platform (R Development Core Team, 2008).

A multilevel model (McMahon and Diez 2007) was first fitted for each foliar trait (Θ) according to

$$\Theta = \mu + U_S + E + \varepsilon \quad , \qquad (1)$$

where μ represents the dataset mean, U_S is a random effect assuming a different value for each species, E is a (fixed) "environmental" effect assuming a different value for each plot (sites along the chronosequence), and ε is the residual error. All parameters were estimated by the Residual Maximum Likelihood (REML) method with the *lme4* library (Bates and Sarkar 2007). The multilevel model Eq. (1) can be used to estimate group- or individual-level regression coefficients and their variation in unbalanced datasets (Gelman & Hill, 2006; p. 246) with even one observation per group (Gelman & Hill, 2006; p. 276).

As described in detail by Fyllas *et al.* (2009), the hierarchical model is able to adequately extract both the variance structure and the magnitude of the species/plot effects. Most importantly, it also provides unbiased estimates of the slopes of the bivariate relationships existing between the various traits of interest for both the genetic and plot-environmental effects. The derived environmental term is considered to represent the combined influences of climate, soil and location. The genetic term represents the species effect. For the estimation of representative plot-level values (taking into account variations in community composition), for each plot/trait combination we determined a species-abundance weighted mean trait value, $\langle\Theta\rangle_P$, simply calculated as

$$\langle\Theta\rangle_P = \frac{\sum_{S=1}^{N} n_S \Theta_S}{\sum_{S=1}^{N} n_S} \quad , \qquad (2)$$

where Θ_S is the mean value of trait in question observed for species *S* within the plot (P); n_S represents the absolute abundance (stem density, which for the most abundant species equates with relative cover/biomass) of species *S* in that plot (unpublished data, Sarah Richardson, Landcare Research, New Zealand) and with *N* the total number of species sampled in each plot. The associated species-abundance standard deviation was also determined for each trait/plot combination using standard formula as applied in the SDMTools package available within R.

From Eq. (1) an "intrinsic" trait value for each species (Θ_G) can be estimated as equal to $\mu + U_S$; this representing the mean value that would be observed should that species be distributed equally across all plots (or from another viewpoint - the trait value predicted to be observed should there have been some sort of "average plot" for which $U_P = 0$). Ignoring the residual term in Eq. (1), it then follows that because

$$\frac{\sum_{S=1}^{N} n_S(\mu + U_S + E)}{\sum_{S=1}^{N} n_S} = \frac{\sum_{S=1}^{N} n_S(\mu + U_S)}{\sum_{S=1}^{N} n_S} + \frac{\sum_{S=1}^{N} n_S(E)}{\sum_{S=1}^{N} n_S} \quad , \qquad (3a)$$

as all species are subject to the same environmental effects (i.e. there is no interaction between U_S and *E* in Eq. 1), it further follows that

$$\langle\Theta\rangle_{\mathrm{P}} = \mu + \frac{\sum_{S=1}^{N} n_{\mathrm{S}}(U_{\mathrm{G}})}{\sum_{S=1}^{N} n_{\mathrm{S}}} + E \quad . \qquad (3b)$$

With $\langle\Theta\rangle_{\mathrm{G}}$ representing the species abundance weighted U_{G}, Eq. 3b can then be simply expressed as

$$\langle\Theta\rangle_{\mathrm{P}} = \mu + \langle\Theta\rangle_{\mathrm{G}} + E \quad . \qquad (3c)$$

In practice the estimation of $\langle\Theta\rangle_{\mathrm{G}}$ using the equation (1) derived U_{S} is slightly problematic due to the random effects in the mixed model fit being quantified through the best linear unbiased predictor (BLUP) method: this giving rise to shrunken estimates of the differences between terms and the overall means (Galwey 2006). We thus simply estimate $\langle\Theta\rangle_{\mathrm{G}}$ (as for example in a slightly modified way here in Figs 2-4) as the difference between $\langle\Theta\rangle_{\mathrm{P}}$ and $(\mu + E)$.

Bivariate relationships of foliar properties were first assessed with Pearson's correlation coefficient (r), and with Standardised Major Axis (SMA) line fits (Warton *et al.* 2006) subsequently applied where r was significantly different from zero. SMA regression lines represent the first axis of a principal component analysis (of a correlation matrix) and are often used in plant allometry studies. It is common for variables to be logarithmically transformed with the regression $\log(y)=\log(\beta)+\alpha\log(x)$, this expressing a power law of the form $y=\beta x^{\alpha}$. SMA regressions were used for both the genetic and environment components of different trait pairs.

Results

Partitioning of variance

Individual species/site (plot) data were presented previously (Atkin *et al.* 2013) and are reproduced in Table S1. Partitioning of the variance in foliar traits into genetic (species) and environmental (plot-level) components is presented in Fig. 1. This shows that the proportion of the variance attributable to the species component differs for different traits. For example, for M_{A} the species component accounted for approximately 87% of the total variance, with the variability associated with site, the environmental effect, being only 3% of the total variance. Approximately 10% of the variance in the dataset was attributable to an error term that

represents the proportion of the variance attributable to intra-species variability as well as any measurement error. Similarly, N_M was more strongly influenced by species (64%) than by site (22%). By contrast, the principal source of variation in P_M was site (accounting for 44% of the total variance) with only 34% of the observed variance attributable to species. For both N and P, the variance of area-based measures (N_A and P_A) had a similar site component but a larger residual component. Variance in the N:P ratio was explained primarily by site (45%) and error (41%) effects.

Variance in gas exchange traits was explained to a greater extent by species than by site (Fig. 1), although error effects also contributed significantly to variance. In both A and R, on a mass-, N- and P-basis, variance was explained more by species than by site. This was particularly so for A_M and R_M, where species accounted for 80% and 77% of total variance, respectively. This contrasted with A_A and R_A, in which variance explained by species was 47% and 28%, respectively, although variance was still explained much more strongly by species than by site. Variance in leaf carbohydrate concentration was explained by species and error components, with virtually no explanatory power in site. Variation in leaf soluble sugar concentration was dominated by species (84-87% for glucose, fructose and total sugars; sucrose was somewhat more strongly influenced by E). Variance in starch and total carbohydrate concentration was explained almost equally by species and error components.

Responses to site age

The partitioning of variance to G and E components also allowed us to investigate the underlying relationship between various traits and site age. The major question we asked here was, once you remove the G component, how important are the underlying E effects in influencing intraspecific variation in leaf traits along the chronosequence? In Figures 2-4, this question is addressed by comparing the overall response (raw data, closed symbols) with the environment-only response (G effects removed, open symbols); the latter being estimated as that value of $\langle\Theta\rangle_P$ predicted to be observed from Eq. 3c with $\langle\Theta\rangle_G$ taken as invariant and equal to that estimated at the youngest site (8 years old) within the chronosequence. Referring to the corresponding estimated value as $\langle\Theta\rangle_E$ this thus represents the community-level mean trait value at any particular site if species composition had not changed with ecosystem development. The differences between $\langle\Theta\rangle_P$ and $\langle\Theta\rangle_E$ in Figs 2-4 thus represent $\langle\Theta\rangle_G$ relative

307 to that at the youngest site. That is to say, referring to this as $\langle\Theta\rangle^{*}_{G}$, this represents the
308 modulating effect of chronosequence changes in species composition on the community-level
309 trait averages observed.
310
311 There was a strong increasing trend in raw site-averaged M_A from around 120 g m^{-2} at site 1 to
312 ~320 g m^{-2} at site 6 (Fig. 2; closed symbols). Where individual species were sampled at more
313 than one site, there were generally very small increases in M_A at older sites. Thus, when the *G*
314 component of the response was removed, there was no underlying impact of *E* (Fig. 2; open
315 symbols). Site-averaged [N] on a mass basis (N_M) was greatest at sites 1 and 2 (~22 mg N g^{-1})
316 and lowest (~8 mg N g^{-1}) at site 6 (Fig. 2; closed symbols), but this range of response was more
317 constrained (~22-15 mg N g^{-1}) when the *G* effect was removed. As reflected in the partitioning
318 of variance (Fig. 1), foliage [P] on a mass basis (P_M) and the N:P ratio both had stronger *E*
319 effects than did [N], so that there was relatively little difference between the raw and *E*-only
320 response to site age (Fig. 2).
321
322 Leaf gas exchange traits varied significantly along the Franz Josef Chronosequence. There was
323 also considerable difference in the extent to which the raw and *E*-only responses to site age
324 agreed (Fig. 3). The clearest differences were evident when comparing the responses of both *A*
325 and *R* on an area- and a mass-basis. A_A and R_A (Fig. 3, first row) displayed similar raw (closed
326 symbols) and *E*-only responses (open symbols). However, because variance in A_M and R_M was
327 explained more by *G* than *E*, the E-only response was much more limited than the raw response
328 (Fig. 3, second row). This more muted *E*-driven response to site age was less evident for A_N
329 and A_P (Fig. 3) and especially R_N and R_P, where there was very little difference between raw
330 and *E*-only responses (and stronger error effects in the case of R_N). As a consequence of the
331 dominance of *G* and error effects on foliar carbohydrate conentrations (Fig. 1), there was a
332 significant difference between raw and *E*-only responses, with virtually no underlying *E*-only
333 site-age impacts (Fig. 4).
334
335 Bivariate relationships between traits
336 A preliminary analysis of foliar traits showed significant correlations between many trait pairs
337 (summary of trait pairs examined here in Table 1, complete listing in Table S2). Where
338 significant correlations existed, further SMA regression analysis was used to investigate
339 bivariate relationships. In particular, we aimed to identify differences in regression estimates

between overall (raw) responses (typical of most previous analyses of this type) and *G*-only responses. These separate bivariate log-log comparisons are shown in Figs. 5, 6 and Fig. S1 and are detailed below. The regression statistics for these relationships are shown in Table 2. A significant point of overall interest here is the comparison of the SMA slopes between the raw relationships and the *G* component – this describes the functional relationship across and within species respectively (although the "residual" component must also contain some aspects of within-species variability). Out of the 22 cases where we made comparisons between pairwise relationships for the genetic and overall relationships (Table 2), 11 had significantly different relationships. In seven of these cases, the genetic component displayed relationships that were more constrained (flatter slopes) than the raw relationships. In addition, in 21 of the 22 comparisons, r^2 values for the relationships were greater when the *E* component was removed.

Leaf N_M and P_M were strongly associated with $1/M_A$ (specific leaf area), and N_M and P_M were positively associated with each other (Fig. 5). In all three cases, the raw relationship differed significantly from the *G*-only relationship, with flatter slopes and higher intercepts for the latter (Table 2). Leaf N_A and P_A were positively associated with M_A, although the strength of these relationships was weaker and there was no difference between the raw and *G*-only relationships. Area- and mass-based *A* and *R* also showed significant relationships with leaf structure (M_A or $1/M_A$ as appropriate), [N] and [P] (Figure 6). A_A was negatively associated with M_A and with N_A. There was no association between A_A and P_A. The *G*-only relationship between A_A and M_A had a slope which was significantly lower than the raw data relationship (Table 2). This was also the case for the A_A-N_A relationship. Relationships between A_M and both $1/M_A$ and N_M were strongly positive but were identical for raw and *G*-only comparisons. The relationship between A_M and P_M was strongly positive and once again, the *G*-only relationship had a slope that was significantly flatter than the raw data relationship (Table 2).

R_A was negatively associated with M_A and weakly associated with P_A but was not significantly associated with N_A. The *G*-only relationship with M_A had a slope that was significantly flatter than the raw data relationship (Fig. 6; Table 2). R_M was positively associated with $1/M_A$, N_M and P_M. The raw and *G*-only relationships between R_M and $1/M_A$ did not differ, but in the relationships between R_M and N_M and P_M the raw and *G*-only relationship differed, and in both cases the *G*-only relationship had a steeper slope than the raw relationship. Finally, while we found significant correlations between total sugars and $1/M_A$, N_M and P_M (Table 1), SMA

regression analysis revealed weak raw relationships, no significant relationships for the *G* component, and no significant differences between raw and *G* regression statistics (Table 2 and Fig. S1). This was also the case for the relationships between R_M and total sugar concentration and starch concentration and A_M.

Discussion

In this study, we present a broad range of leaf functional traits in the most dominant species in temperate rainforest communities along a 120,000-year soil-development chronosequence. Because there are instances where species were sampled at multiple sites, an important aspect of the present study is that we were able to statistically partition (Fyllas *et al.* 2009) the influence of species (*G*) and environment (*E* i.e. soil characteristics) on changes in a broad suite of leaf functional traits over this very steep nutrient gradient. This approach allows us to broaden the interpretation of biological responses in gradient studies. Importantly, and in support of our first hypothesis and niche-based community ecology, we found that when accounting for differences in plot-level trait averages ($\langle\Theta\rangle_P$), changes in species composition ($\langle\Theta\rangle_G^*$) may account for greater variability than effects of substrate age (as expressed through $\langle\Theta\rangle_E$). However, this varied significantly among traits. This has important implications for our understanding of ecological drivers of plant functional traits.

The importance of species and environment to trait variation

Previous analyses of global (Wright *et al.*, 2004) and regional (Fonseca *et al.* 2000; Wright *et al.* 2001) datasets have suggested that a large portion of the variation observed in leaf traits may be found between species within a common environment (thus driven by *G*), with Townsend *et al.* (2007) highlighting the importance of local-scale species diversity as an important component controlling the variation of foliar N:P ratio. This contrasts with the notion that, if an element is limiting for plant growth, then foliar concentrations should show a strong correlation with variation in its availability within the soil. Resolution of the question of trait variation across species and environments is not helped by the fact that traits are generally measured on single estimates per species [potentially ignoring intraspecific variation - (Albert *et al.* 2011; Bolnick *et al.* 2011)], or are insufficient to separate changes in trait values from changes in the composition of species across gradients (Wright *et al.,* 2005). However, despite

406 recognition that there is likely merit in differentiating between *G* and *E* effects (Wright *et al.*
407 2005b), explicit quantification of the relative importance of the *G* and *E* components of
408 variation in traits has been attempted only relatively recently. In a glasshouse-based study
409 investigating wetland species responses to water table depth and N availability, Wright &
410 Sutton-Grier (2012) found significant species by environment interactions for leaf traits (M_A,
411 leaf [N], photosynthesis rates). Fyllas *et al.* (2009), using a statistical approach that informed
412 the current study, reported on the partitioning of variance in leaf traits for tropical forest
413 species. This showed that properties like M_A, and leaf [N], [Mg] and [Al] were more strongly
414 constrained taxonomically, while others ([P], [Ca] and [K]) were more strongly controlled by
415 environment. Dahlin *et al.* (2013), using remote sensing techniques, found that some leaf traits
416 (e.g. N_M and C_M) are more strongly predicted by community composition than environment
417 across a diverse Mediterranean-type ecosystem.
418
419 In our field study, we present findings that illuminate the above mentioned patterns in
420 temperate rainforest communities responding to soil development. Leaf mass per unit area (M_A)
421 increased by a factor of three between the youngest and oldest sites, but was strongly influenced
422 by species identity (Fig. 1), so that when the *G* component of these traits was removed, the
423 underlying *E* component of the response to site age was non-existent (Fig. 2). Although M_A
424 increased considerably with soil age (and thus increased nutrient limitation), the lack of an *E*-
425 only impact on M_A demonstrates a strong relative taxonomic constraint on phenotypic plasticity
426 in leaf structural traits. This is consistent with previous analyses (Poorter *et al.* 2009), but here
427 our measurement of leaf carbohydrate concentration (which also has no *E*-only response, as
428 discussed below) also provides strong evidence that accumulation of non-structural
429 carbohydrates under nutrient limitation is *not* a driving mechanism for differences in M_A. We
430 are left to conclude that differences in M_A arise from inherent species differences in leaf
431 thickness and density.
432
433 The relatively weak *E*-only response of leaf [N] clearly shows a partial role for community
434 composition in influencing nutrient cycling. By contrast, foliar [P] had large variance that was
435 partitioned roughly equally between *G* and *E*. Importantly, variation in the N:P ratio was driven
436 by *E*, so the response to soil age was very strong even with the *G* component removed. The
437 paucity of previous studies that explicitly quantify both N and P nutrition *and* partition variance
438 provide little evidence against which to judge this striking result. In previous extensive
439 sampling of species at sites along the chronosequence, Richardson *et al.* (2004) also showed

that there was a general increase in the ratio of N to P concentration in leaves at soils with increasing age. They noted that this may not be solely related to soil nutrient availability because of the potentially higher tolerance of conifers to low P supply compared with angiosperms at the older sites. But our results indicate that species identity is unlikely to be an explanatory variable in this response along the chronosequence. The strong *E* effect on foliar N:P is consistent with recent findings for roots (Holdaway *et al.* 2011), and provides evidence from low nutrient availability temperate rainforest that contradicts the idea from other forest (Townsend *et al.* 2007) and grassland (He *et al.* 2008) systems that the dominant influence on foliar N:P ratios is species variability. Here we conclude that, while phylogeny/taxonomy have some role in shaping leaf [N] (Niklas *et al.* 2005), leaf [P] and N:P values display strong phenotypic plasticity and are strongly influenced by environment, especially in the older, low-P communities.

Area-based measures of leaf gas exchange (*A* and *R*) displayed variance that was dominated by *E* and error, with mass-based measures more strongly influenced by *G* (Fig. 1). This clearly reflects the strong role that *G* plays in determining leaf M_A. As a result, the *E*-only response of A_M and R_M (Fig. 3) was less pronounced than the overall response. The fact that A_A declined significantly across the sequence despite increases in M_A (which could be expected to be a surrogate for leaf thickness/density and potentially metabolic capacity) likely indicates a greater proportion of non-metabolic cell types contribute to M_A in the older sites (Hikosaka and Hirose 2000; Wright *et al.* 2005a). Variance in element-based measures of *A* was explained less strongly by *G*, so the *E*-only response sat closer to the overall response than did mass-based measures. Consistency in R_N and R_P across the chronosequence indicates a strong coupling between factors influencing respiration and factors affecting foliage N and P concentration. We conclude from these results that differences in dark respiration are likely based upon a tight coupling between site soil nutrient availability and stand growth rate and demand for energy associated with foliage maintenance (Lambers *et al.* 1998; Turnbull *et al.* 2005). The extent of the error term in area-based measures of *A* and *R* (Fig. 1) is also important – this variance is an expression of the recent physiological past of experimental plants (e.g. acclimation to changes in environment) in addition to experimental error.

Leaf carbohydrate concentrations provide a potential barometer of source/sink activity in response to environment. We might expect *A* to decrease to a lesser extent than growth/sink (and hence *R*) activity in response to soil nutrient depletion, which would result in leaf

474 carbohydrate accumulation (Poorter *et al.* 2009), although to our knowledge this has never
475 been comprehensively tested under field conditions. We found that starch concentration was
476 broadly unresponsive to site age, while soluble sugar concentration became progressively
477 reduced in the nutrient-poor older sites (Fig. 4). With each of these contributing roughly equally
478 to total non-structural carbohydrate concentration, this resulted in no evidence for
479 accumulation. The response of total soluble sugars suggests that assimilation rates are so low
480 in trees in the nutrient-poor older sites that the level of metabolic activity is a strong enough
481 sink to deplete them. In addition, the systematic differences that we did observe in leaf
482 carbohydrate concentrations were overwhelmingly explained by *G*. Starch and TNC had a
483 weaker *G* component and stronger residual error component than component sugars (but still
484 with no *E* effect). This indicates that species identity is the primary determinant of patterns of
485 carbohydrate concentration across this chronosequence.
486
487 Ultimately, the most important implication of these findings is that both *G* and *E* drivers of
488 variation must be considered when interpreting trait shifts in field data. This highlights the
489 inherent challenge recently raised by Poorter *et al.* (2013) in relation to trait correlation
490 networks – to establish the extent to which variables are affected by a given environmental
491 factor and then whether the same network topology exists when plants are all grown under
492 common conditions. Analysis of plant responses along gradients may often involve both
493 environmental and taxonomic changes, and it is essential that both are recognized explicitly in
494 broad-scale analyses. Clearly, ecological sorting (*sensu* Ackerly 2003) accounts for the
495 differences in species composition observed along the Franz Josef Chronosequence. In this
496 context, we see a strong *G* component to variation in a number of structural and physiological
497 traits. Of course, in a global ecological sense, *G* and *E* are not mutually exclusive. For example,
498 species on richer soils tend to have intrinsically lower M_A, and intrinsically higher leaf nutrient
499 concentrations compared with species on poor soils. This shift along the axis of species
500 variation represents "habitat tracking" (Ackerly 2003; Lusk *et al.* 2013) and shows that specific
501 trait dimensions systematically change along soil fertility gradients. It confirms the idea that,
502 although individual plasticity in traits may be limited, maximum response at the
503 community/landscape level may be achieved by genetic differences between species (Gleason
504 *et al.* 2009). It also provides evidence to support the suggestion that predictions of the full
505 response of traits across environmental gradients (or to environmental change) may only be
506 achieved via changes in species composition (Wright and Sutton-Grier 2012; Hayes *et al.*
507 2014).

508

509 Implications for analysis of bivariate relationships

510 Although we know the slopes of the relationships between leaf traits may vary in response to
511 water and nutrient availability (Wright *et al.* 2001), the significance of within-species
512 variability relative to plot changes in species composition is not clear (Albert *et al.* 2011;
513 Wright and Sutton-Grier 2012). This is important, as a significant proportion of the overall
514 response of the flora in this temperate rainforest is actually via ecological sorting during
515 ecosystem progression and retrogression. Relationships between leaf chemical traits (Figs. 5
516 and 6; Table 2) generally follow power functions, with presentation on a log-log basis
517 linearizing the relationships, and nearly all relationships are allometric rather than isometric
518 (i.e. all slopes are significantly divergent from 1). Importantly, relationships are maintained
519 when the *E* component of these relationships is removed. However, in a number of cases, SMA
520 tests supported our second hypothesis, and revealed significant differences in slopes and
521 intercepts between raw and *G*-only relationships, with 11 of the 22 relationships displaying *G*-
522 only relationships that are more constrained (significantly lower slopes) than the raw
523 relationships.

524

525 How do our values of the slopes of the bivariate relationships compare with those proposed to
526 operate globally (Wright *et al.* 2004), and how much of this results from *G* rather than *E*
527 effects? For raw trait variation we observed N_M-1/M_A slopes of 0.77, almost identical to that of
528 Wright *et al.* (2004), who (taking reciprocal values from M_A-N_M relationships in their Table 1)
529 reported a value of 0.78 (Table 2, final column). However, this slope decreased to 0.54 for the
530 *G*-only component of trait variation. Similarly, our raw scaling slope for P_M-1/M_A of 1.18 is
531 very close to the Wright *et al.* (2004) global estimate of 1.22, but this decreased to 0.63 when
532 the *E* component is removed to leave the impact of species identity. This pattern was replicated
533 for area-based comparisons of N and P with M_A, which also broadly conformed with global
534 averages, although the N_A-M_A relationship did not differ between raw and *G*-only relationships.
535 In contrast to these chemical trait relationships, those describing the relationships between
536 carbon exchange parameters and foliar chemistry differed from the global estimates of Wright
537 *et al.* (2004). For raw trait variation we observed an A_M-1/M_A slope of 2.03, steeper than the
538 value of 1.33 given by Wright *et al.* (2004). Similarly, the A_M-N_M slope of 2.74 was steeper
539 than the global value of 1.72, as was the A_M-P_M relationship (slope of 1.82, steeper than the
540 global value of 1.03, and this slope increased to 2.87 for the *G*-only relationship). The raw
541 scaling slope for R_M-1/M_A of 1.48 was greater than the global estimate of 1.05, as was the R_M-

542 N_M slope [1.99 cf. global value of 1.43, and this slope increased to 2.45 for the *G*-only
543 relationship (at P=0.08)] and the R_M-P_M relationship (slope of 1.32, global value 0.96, 2.12 for
544 the *G*-only relationship). The generally steeper slopes of scaling relationships between carbon
545 exchange and leaf chemical traits in the present study compared with global values is likely
546 indicative of a more constrained range of values. Overall, these findings suggest strong
547 similarities in the overall response of leaf chemical traits between temperate rainforest trees
548 and other terrestrial plants. However, we found that the relationships between *A* and *R* and
549 foliar traits in these species do not conform to global averages. Importantly, our analysis shows
550 that a large proportion of trait-trait relationships may be species based, and not a plastic
551 response to environment *per se*. This needs to be considered when using such relationships in
552 order to make predictions of likely responses of plants to environmental change. Interestingly,
553 in all of the bivariate comparisons, we also found that r^2 values were greater in the *G*-only
554 relationships (i.e. with the *E* effect removed) – this suggests that much of the scatter in reported
555 bivariate relationships could be due to the impact of *E* on leaf traits (e.g. extent to which
556 available N and P are allocated to metabolism versus structural components in leaves).
557
558 ## Conclusions
559 This study contributes to the assessment of factors controlling variability in plant traits over
560 wide spatial scales. The extent to which foliar traits are more heavily influenced by *G* or *E*
561 depends on the foliar property: some leaf traits are more phylogenetically constrained (M_A, leaf
562 carbohydrate concentration), while others display a strong of phenotypic plasticity after the *G*
563 component has been removed; traits such as foliar [N]:[P] show strong associations with
564 growing conditions that are likely linked to variations in stand-level productivity.
565 Environmental effects on leaf level nutrient concentrations make the use of general scaling
566 relationships for *A* and *R* difficult, especially if soil fertility variations are not implicitly taken
567 into account. Conversely, the strong *G* correlations between M_A and leaf nutrient
568 concentrations confirms the general existence of physico-chemical leaf trait relationships
569 across the diverse group of tree species studied here (and in other studies e.g. Fyllas *et al.*
570 2009). Thus, when considering how changes in nutrient availability along soil chronosequences
571 impact on leaf traits, care is needed when assessing the 'direct' effects of altered N and P
572 availability on those traits, given the predominant role that changes in species composition
573 plays in controlling trait variation along the chronosequence, with plant species associating
574 with the soil conditions most appropriate for their growth. While this finding is perhaps not
575 surprising (given that species follow distribution patterns based on an association between

dispersal limitations, genetic trait potential and the availability of environmental resources), our study is one of the few to quantify the relative importance of genetic and environmental variables in controlling trait variation under field conditions.

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827

828 **Table 1.** Correlations between leaf traits sampled along the Franz Josef Glacier soil
829 chronosequence. Abbreviations: M_A - leaf dry mass per unit area; $P_{A/M}$ and $N_{A/M}$ - leaf
830 phosphorous and nitrogen concentrations on per unit area and per unit mass,
831 respectively; $A_{A/M/N/P}$ – assimilation rate at saturating light on an area, mass, N and P
832 basis, respectively; $R_{A/M/N/P}$ – leaf dark respiration rate on an area, mass, N and P
833 basis, respectively. Pearson coefficients (*r*) and significance values (*P*) for each correlation
834 are shown.
835
836

Trait (log)	Trait (log)	*r*	*P*
N_A (g m^{-2})	M_A (g m^{-2})	0.676	**<0.001**
P_A (g m^{-2})	M_A (g m^{-2})	0.174	**<0.05**
P_M (mg g^{-1})	N_M (mg g^{-1})	0.885	**<0.001**
N_M (mg g^{-1})	1/M_A (m^2 kg^{-1})	0.764	**<0.001**
P_M (mg g^{-1})	1/M_A (m^2 kg^{-1})	0.695	**<0.001**
A_A (μmol m^{-2} s^{-1})	M_A (g m^{-2})	-0.421	**<0.001**
A_A (μmol m^{-2} s^{-1})	N_A (g m^{-2})	-0.189	**<0.05**
A_A (μmol m^{-2} s^{-1})	P_A (g m^{-2})	0.023	**ns**
A_M (nmol g^{-1} s^{-1})	1/M_A (m^2 kg^{-1})	0.598	**<0.001**
A_M (nmol g^{-1} s^{-1})	N_M (mg^{-1} g^{-1})	0.604	**<0.001**
A_M (nmol g^{-1} s^{-1})	P_M (mg^{-1} g^{-1})	0.638	**<0.001**
R_A (μmol m^{-2} s^{-1})	M_A (g m^{-2})	-0.236	**<0.01**
R_A (μmol m^{-2} s^{-1})	N_A (g m^{-2})	-0.015	**ns**
R_A (μmol m^{-2} s^{-1})	P_A (g m^{-2})	0.212	**<0.05**
R_M (nmol g^{-1} s^{-1})	1/M_A (m^2 kg^{-1})	0.626	**<0.001**
R_M (nmol g^{-1} s^{-1})	N_M (mg g^{-1})	0.706	**<0.001**
R_M (nmol g^{-1} s^{-1})	P_M (mg g^{-1})	0.749	**<0.001**
Tot Sugars (mg g^{-1})	1/M_A (m^2 kg^{-1})	0.437	**<0.001**
Tot Sugars (mg g^{-1})	N_M (mg g^{-1})	0.418	**<0.001**
Tot Sugars (mg g^{-1})	P_M (mg g^{-1})	0.334	**<0.001**
R_M (nmol g^{-1} s^{-1})	Tot Sugars (mg^{-1} g^{-1})	0.263	**<0.01**
Starch (mg^{-1} g^{-1})	A_M (nmol g^{-1} s^{-1})	-0.218	**<0.05**

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Table 2. Standardized Major Axis (SMA) regression slopes and *y*-axis intercepts for log-log linear relationships between leaf traits sampled along the Franz Josef Glacier soil chronosequence, as shown in Figures 5, 6 and S1. Analysis is separated into pairwise relationships for raw data (R) and genetic (G) effects. Abbreviations: M_A - leaf dry mass per unit area; $P_{A/M}$ and $N_{A/M}$ - leaf phosphorous and nitrogen concentrations on per unit area and per unit mass, respectively; $A_{A/M/N/P}$ – assimilation rate at saturating light on an area, mass, N and P basis, respectively; $R_{A/M/N/P}$ – leaf dark respiration rate on an area, mass, N and P basis, respectively. Coefficient of determination (r^2), significance of relationship (P), slope, y-axis intercept and significance of difference between R and G relationships [P (R vs G)] for each bivariate relationship are shown. *In the final column, the slopes of global relationships published in Wright *et al.* (2004) are shown for comparison (na denotes not available).

y-axis (log)	*x*-axis (log)	Data	*n*	r^2	*P*	Slope	*y*-axis intercept	*P* (R vs G)	Slope (Global)*
N_M (mg^{-1} g^{-1})	$1/M_A$ (m^2 kg^{-1})	R	127	0.750	<0.001	0.770	2.780	<0.05	0.78
		G	16	0.780	<0.001	0.540	2.280		
P_M (mg^{-1} g^{-1})	$1/M_A$ (m^2 kg^{-1})	R	127	0.610	<0.001	1.180	2.520	<0.01	1.22
		G	16	0.620	<0.001	0.630	1.260		
N_A (g m^{-2})	M_A (g m^{-2})	R	127	0.430	<0.001	0.511	-0.830	ns	0.65
		G	16	0.790	<0.001	0.512	-0.830		
P_A (g m^{-2})	M_A (g m^{-2})	R	127	0.012	ns			<0.01	0.81
		G	16	0.346	<0.05	0.338	-1.62		
P_M (mg^{-1} g^{-1})	N_M (mg^{-1} g^{-1})	R	127	0.720	<0.001	1.510	-1.710	<0.05	na
		G	16	0.800	<0.001	1.150	-1.360		
A_A (mol m^{-2} s^{-1})	M_A (g m^{-2})	R	123	0.245	<0.001	-1.352	3.722	0.06	ns
		G	16	0.374	<0.05	-0.872	2.648		
	N_A (g m^{-2})	R	123	0.055	<0.05	-2.694	1.568	0.07	1.21
		G	16	0.273	<0.05	-1.705	1.228		
	P_A (g m^{-2})	R	123	0.018	ns			ns	0.66
		G	16	0.142	ns				
A_M (nmol g^{-1} s^{-1})	$1/M_A$ (m^2 kg^{-1})	R	112	0.668	<0.001	2.032	6.039	ns	1.33
		G	16	0.814	<0.001	1.800	5.519		
	N_M (mg^{-1} g^{-1})	R	112	0.591	<0.001	2.747	-1.461	ns	1.72
		G	16	0.682	<0.001	3.312	-2.025		
	P_M (mg^{-1} g^{-1})	R	127	0.545	<0.001	1.821	1.646	<0.05	1.03
		G	16	0.548	<0.01	2.866	1.881		

y-axis (log)	*x*-axis (log)	Data	*n*	r^2	*P*	Slope	*y*-axis intercept	*P* (R vs G)	Slope (Global)
R_A (μmol m^{-2} s^{-1})	M_A (g m^{-2})	R	108	0.125	<0.001	-0.845	1.854	<0.01	0.83
		G	16	0.263	<0.05	-0.386	0.825		
	N_A (g m^{-2})	R	108	0.009	ns			ns	1.12
		G	16	0.072	ns				
	P_A (g m^{-2})	R	108	0.059	<0.05	1.258	1.079	ns	0.79
		G	16	0.003	ns				
R_M (nmol g^{-1} s^{-1})	$1/M_A$ (m^2 kg^{-1})	R	112	0.700	<0.001	1.482	4.066	ns	1.05
		G	16	0.852	<0.001	1.330	3.712		
	N_M (mg $^{-1}$ g^{-1})	R	110	0.647	<0.001	1.990	-1.389	0.08	1.43
		G	16	0.861	<0.001	2.447	-1.862		
	P_M (mg $^{-1}$ g^{-1})	R	110	0.600	<0.001	1.319	0.862	<0.01	0.96
		G	16	0.779	<0.001	2.118	1.024		
Tot Sugar (mg $^{-1}$ g^{-1})	$1/M_A$ (m^2 kg^{-1})	R	127	0.136	<0.001	0.793	3.533	ns	na
		G	16	0.288	ns				
	N_M (mg $^{-1}$ g^{-1})	R	127	0.111	<0.001	1.026	0.671	ns	na
		G	16	0.226	ns				
	P_M (mg $^{-1}$ g^{-1})	R	127	0.083	<0.001	0.678	1.833	<0.05	na
		G	16	0.288	ns				
R_M (nmol g^{-1} s^{-1})	Tot Sugar (mg $^{-1}$ g^{-1})	R	112	0.079	<0.01	1.972	-2.742	ns	na
		G	16	0.288	ns				
Starch (mg $^{-1}$ g^{-1})	A_M (nmol g^{-1} s^{-1})	R	112	0.103	<0.001	-0.488	2.414	ns	na
		G	16	0.288	ns				

FIGURES

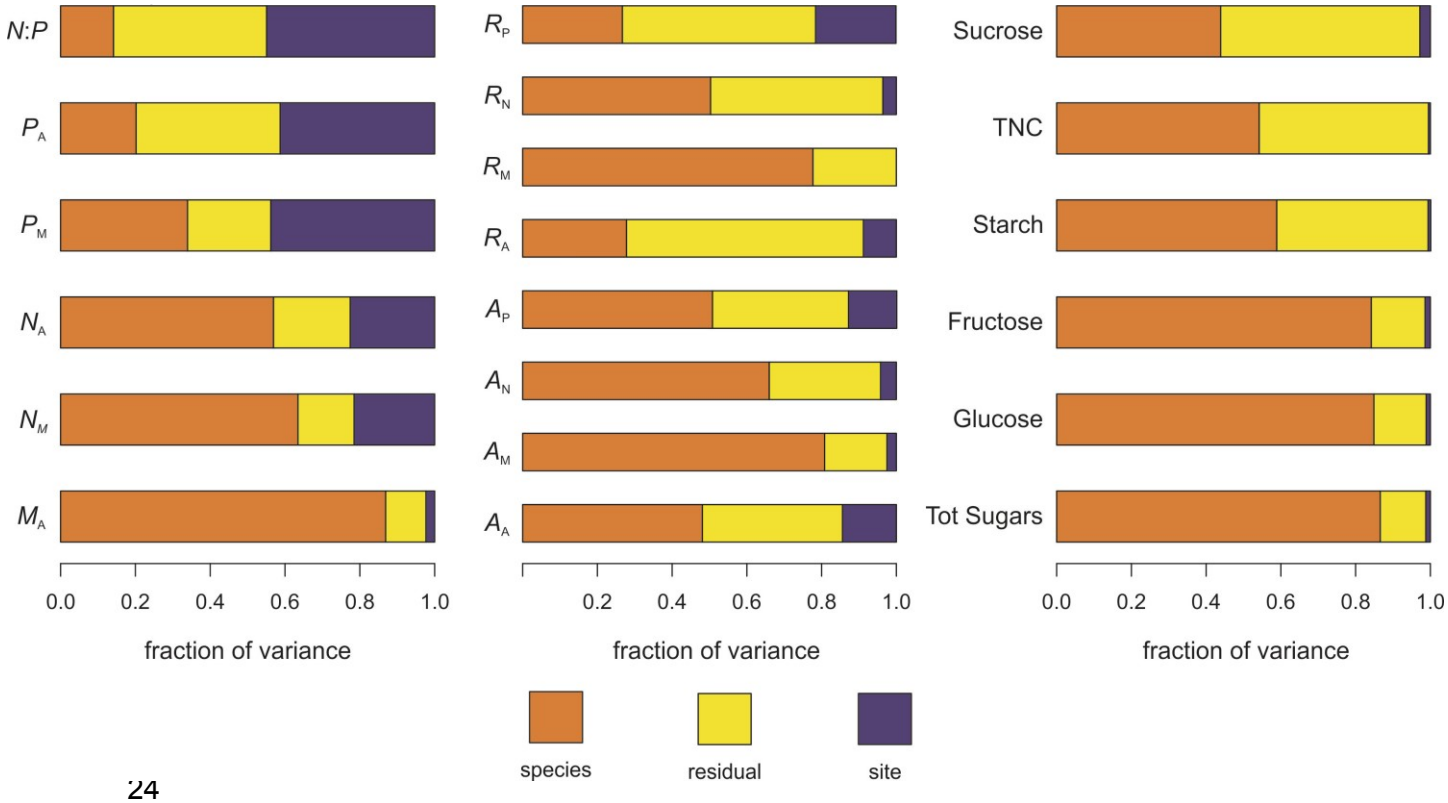

Figure 1. Partitioning of the total variance for each foliar property into species, site and error (residual) components. Abbreviations: M_A - leaf dry mass per unit area; $P_{A/M}$ and $N_{A/M}$ - leaf phosphorous and nitrogen concentrations on per unit area and per unit mass, respectively; $A_{A/M/N/P}$ – assimilation rate at saturating light on an area, mass, N and P basis, respectively; $R_{A/M/N/P}$ – leaf dark respiration rate on an area, mass, N and P basis, respectively; TNC – total non-structural carbohydrate content. For units see Table 1.

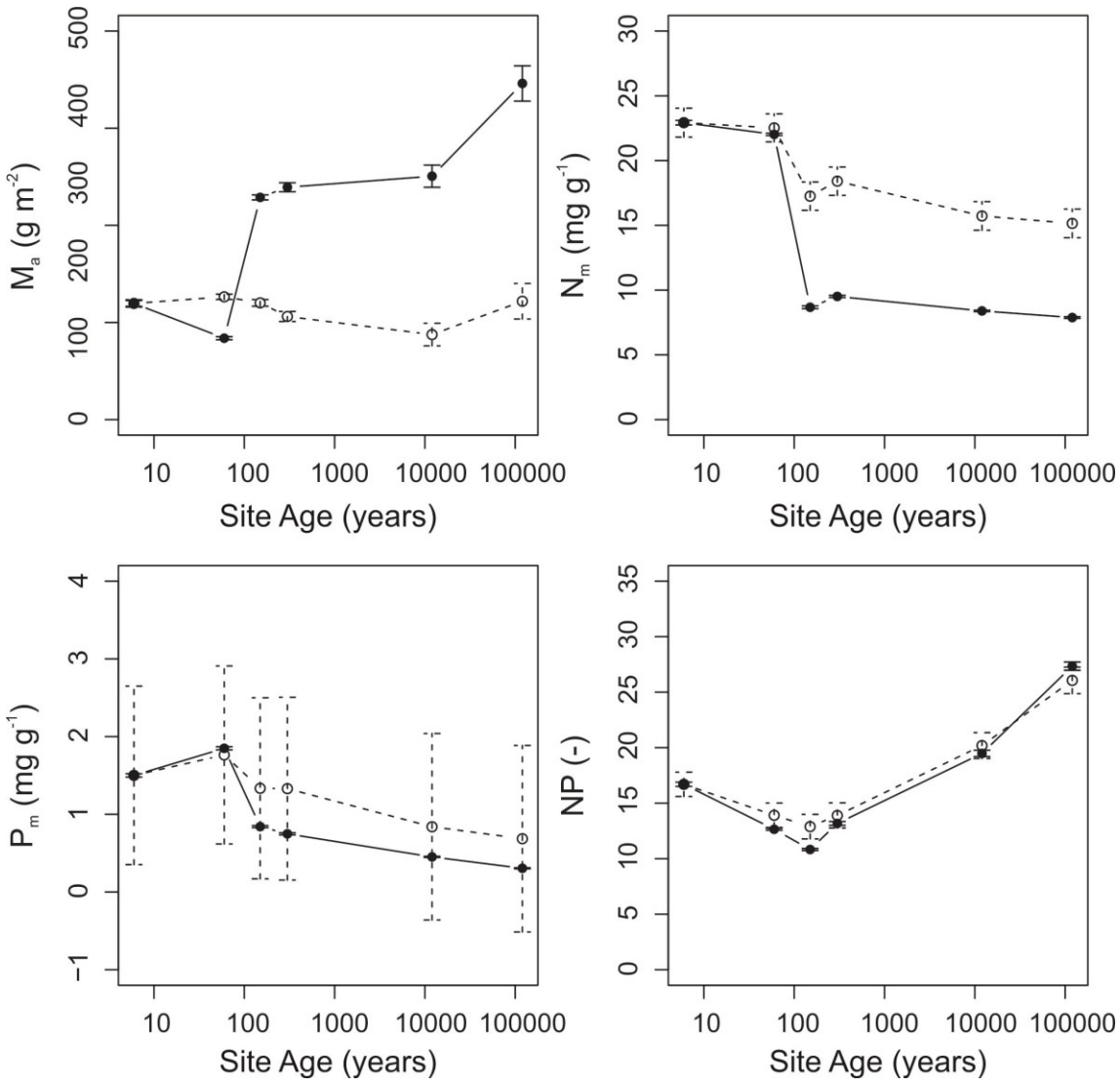

Figure 2. Relationship between leaf structural and chemical traits and site age (on a log scale) for the six study sites sampled along the 120,000 year old Franz Josef Glacier soil chronosequence. Relationships shown for the complete data set (closed symbols) and the environmentally-driven (E) response (i.e. with the G component removed; open symbols). Abbreviations: M_A - leaf dry mass per unit area; P_M and N_M - leaf phosphorous and nitrogen concentrations per unit mass, respectively. Data shown are the mean of 3-7 dominant plant species sampled at each site weighted according to relative abundance in the community (± s.e.). For details of species at each site, see Table S1.

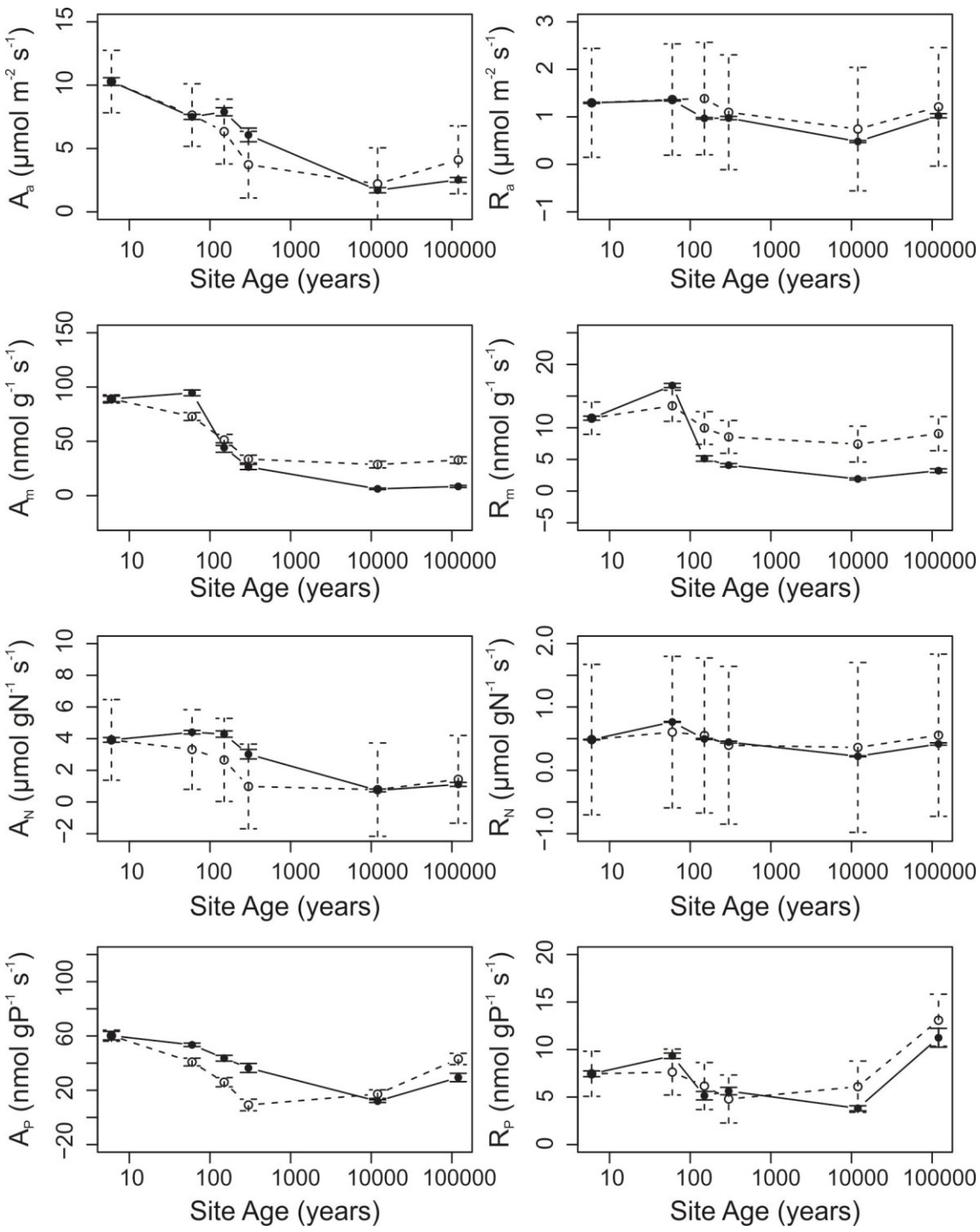

Figure 3. Relationship between leaf gas exchange traits and site age (on a log scale) for the six study sites sampled along the 120,000 year old Franz Josef Glacier soil chronosequence. Relationships shown for the complete data set (closed symbols) and the environmentally-driven (E) response (i.e. with the G component removed; open symbols). Abbreviations: $A_{A/M/N/P}$ – assimilation rate at saturating light on an area, mass, N and P basis, respectively; $R_{A/M/N/P}$ – leaf dark respiration rate on an area, mass, N and P basis, respectively. Data shown

are the mean of 3-7 dominant plant species sampled at each site weighted according to relative abundance in the community (± s.e.). For details of species at each site, see Table S1.

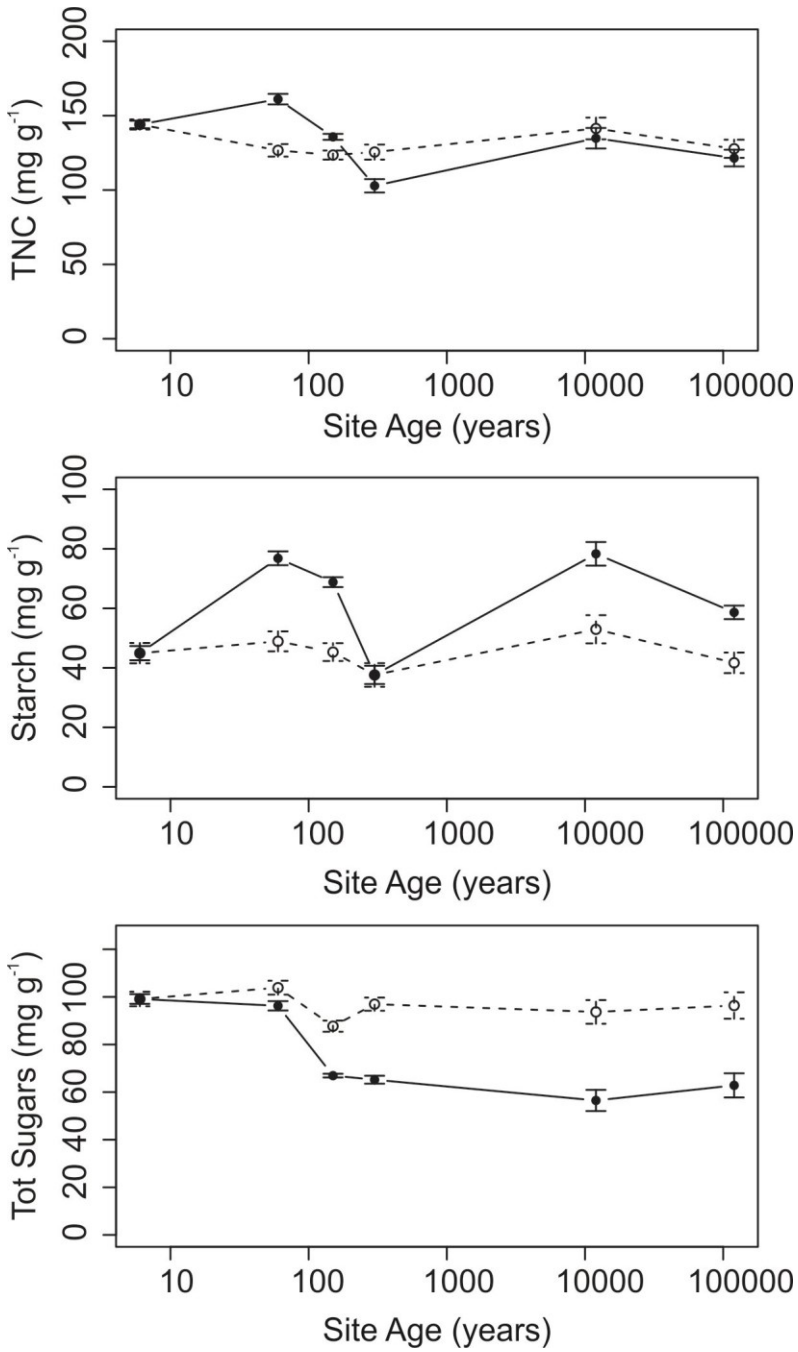

Figure 4. Relationship between leaf carbohydrate concentration and site age (on a log scale) for the six study sites sampled along the 120,000 year old Franz Josef Glacier soil chronosequence. Relationships shown for the complete data set (closed symbols) and the environmentally-driven (E) response (i.e. with the G component removed; open symbols). Data shown are the mean of 3-7 dominant plant species sampled at each site weighted according to relative abundance in the community (± s.e.). For details of species at each site, see Table S1.

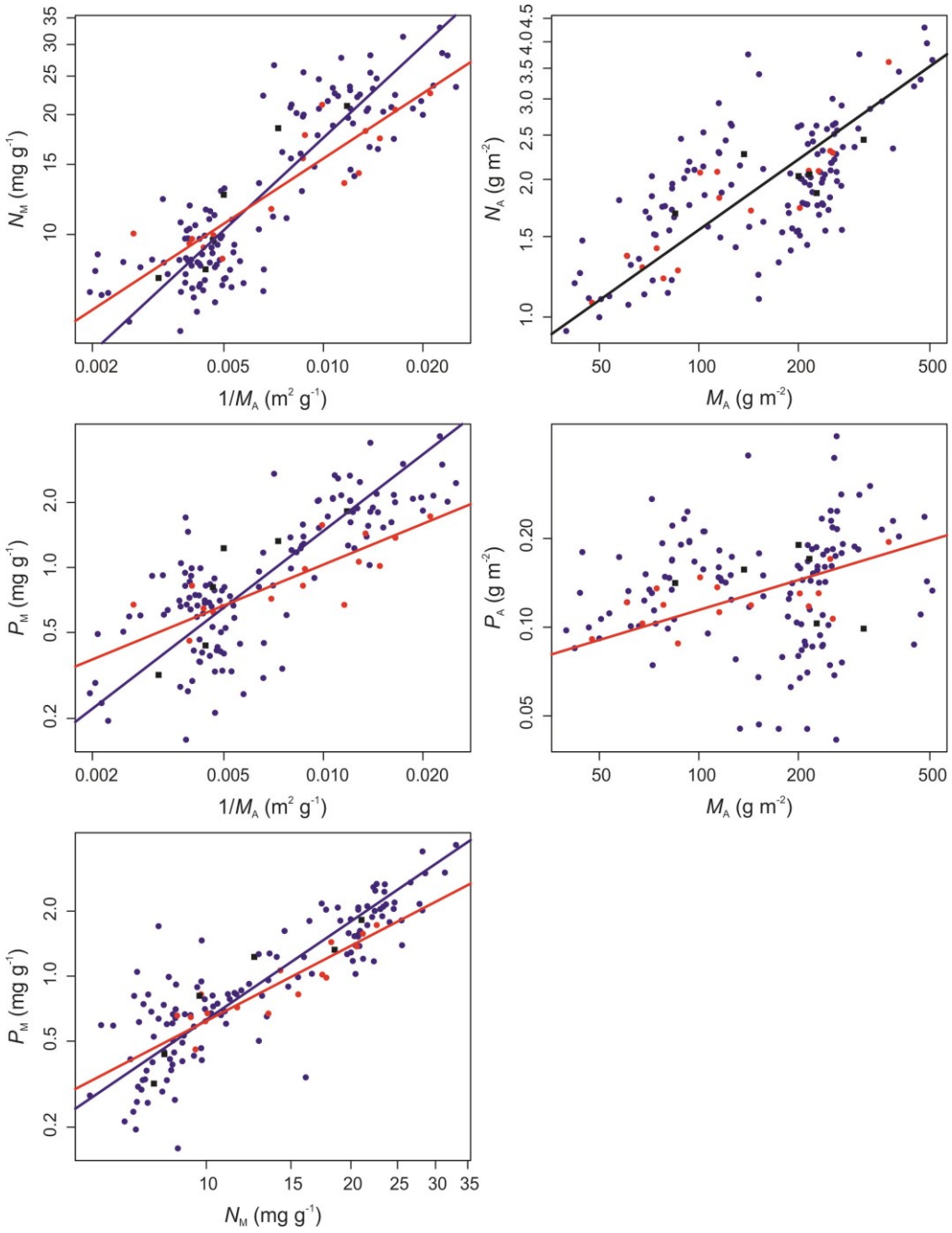

Figure 5. Log-log plots of mass- and area-based leaf chemical traits in relation to leaf structure [mass per unit leaf area (M_A) or leaf area per unit mass ($1/M_A$)] and the relationship between foliar N and P concentrations. Relationships shown for the complete data set (blue symbols), the genetically-driven (G) response (i.e. with the E component removed; red symbols) and for site averages (black squares, not included in regression calculations). Abbreviations: M_A - leaf dry mass per unit area; $P_{A/M}$ and $N_{A/M}$ - leaf phosphorous and nitrogen concentrations on per unit area and per unit mass, respectively. In cases where Standardized Major Axis (SMA) tests for common slopes revealed significant differences between the complete data set and the G relationship (P<0.05), different slopes

and intercepts are provided for each bivariate relationship, otherwise only significant relationships (red for G-only or blue for overall) or a single overall relationship (black line) is shown. See Table 2 for details of bivariate relationships.

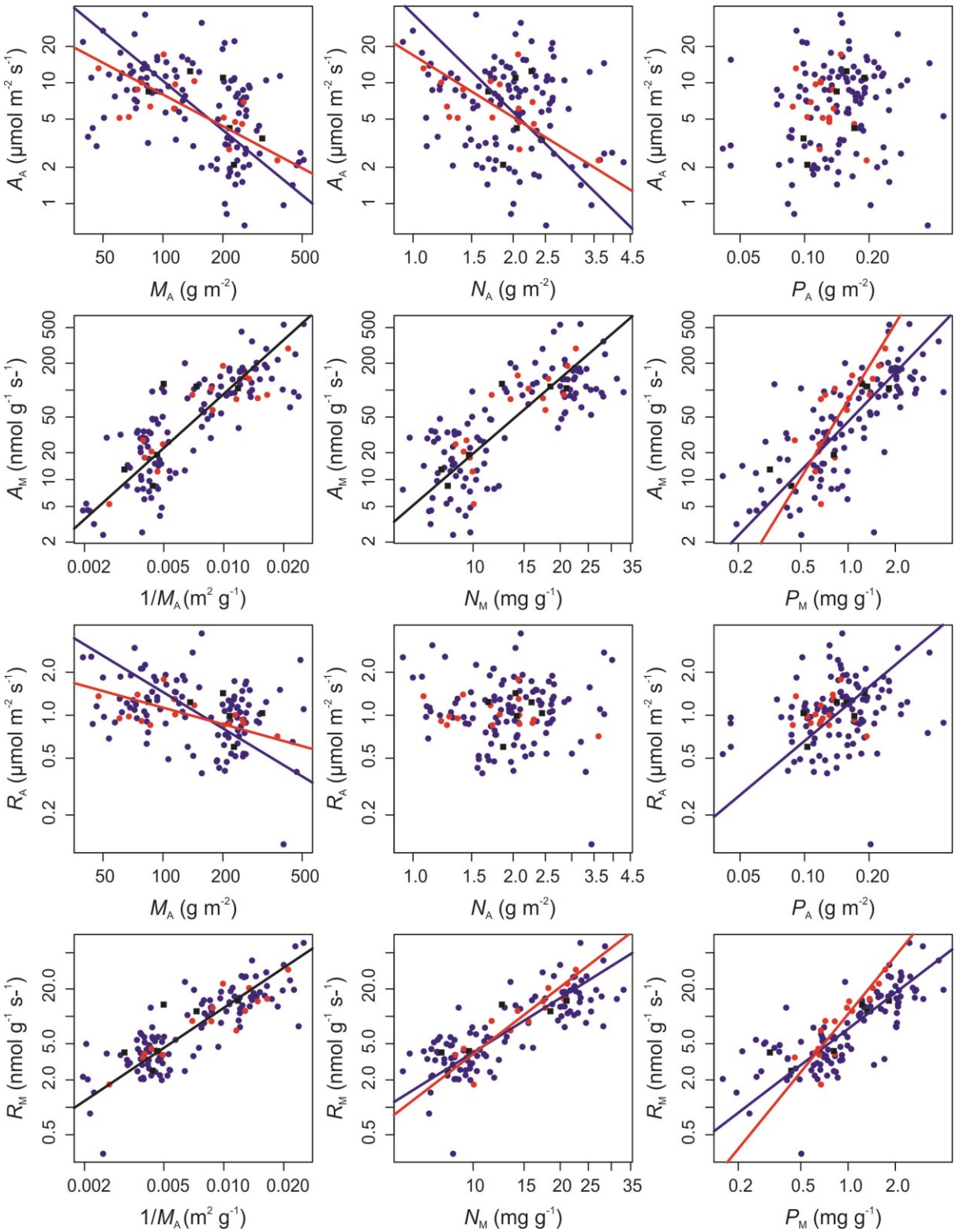

Figure 6. Log-log plots of area- and mass-based leaf gas exchange traits in relation to leaf structure [mass per unit leaf area (M_A) or leaf area per unit mass ($1/M_A$)] and foliar N and foliar P concentrations (on an area or mass basis as appropriate). Relationships shown for the complete data set (blue symbols), the genetically-driven (G) response (i.e. with the E component removed; red symbols) and for site averages (black squares). Abbreviations: M_A - leaf dry mass per unit area; $P_{A/M}$ and $N_{A/M}$ - leaf phosphorous and nitrogen concentrations on per unit area and per unit mass, respectively; $A_{A/M/N/P}$ – assimilation rate at saturating light on an area, mass, N and P basis, respectively; $R_{A/M/N/P}$ – leaf dark respiration rate on an area, mass, N and P

basis, respectively. In cases where Standardized Major Axis (SMA) tests for common slopes revealed significant differences between the complete data set and the G relationship (P<0.05), different slopes and intercepts are provided for each bivariate relationship, otherwise only significant relationships (red for G-only or blue for overall) or a single overall relationship (black line) is shown. See Table 2 for details of bivariate relationships.

Supplementary Material

The following supplementary material is available for this article online:

Table S1 – raw data (previously presented in Atkin *et al.* 2013).
Table S2 – Pearson's correlation matrix
Figure S1 – SMA relationships for leaf carbohydrate bivariate comparisons

Table S1. Average (± s.e., n = 3-5) values of leaf dry mass per unit area (M_A), leaf fresh mass per unit area (F_A), leaf dry matter content (Φ), nitrogen concentration, phosphorus concentration, area-based rates of photosynthesis at 1500 μmol photons m^{-2} s^{-1} PPFD (A), rates of leaf respiration (R), the ratio of leaf R to A, soluble sugar concentration and starch content for each species growing at each site.

Site	Species	M_A (g m^{-2})	F_A (g m^{-2})	Φ (g g^{-1})	N (mg g^{-1})	P (mg g^{-1})	A_A (μmol m^{-2} s^{-1})	A_M (nmol g^{-1} s^{-1})	R_A (μmol m^{-2} s^{-1})	R_M (nmol g^{-1} s^{-1})	Glucose (mg g^{-1})	Fructose (mg g^{-1})	Sucrose (mg g^{-1})	Sugars (mg g^{-1})	Starch (mg g^{-1})
1	*Aristotelia serrata*	68±4	208.7±11.6	0.33±0.01	22.6±0.5	1.8±0.1	12.1±0.5	177.7±5.8	1.4±0.2	20.8±2.2	90.9±6.6	27.5±3.5	5.0±2.6	123.3±5.9	97.8±22.8
(11y)	*Coriaria arborea*	115±14	315.1±10.1	0.36±0.03	24.1±1.3	1.6±0.2	9.5±1.4	84.2±12.0	1.3±0.2	11.7±1.7	82.2±3.2	14.5±1.2	10.2±0.6	106.9±2.8	40.0±10.3
	Griselinia littoralis	232±11	602.1±18.9	0.38±0.01	11.2±0.7	0.9±0.1	8.8±1.0	38.5±5.8	1.0±0.1	4.2±0.6	52.6±3.8	23.0±2.0	4.7±0.8	80.4±5.4	65.7±13.9
	Hebe salicifolia	117±9	333.6±13.5	0.35±0.01	20.1±1.5	1.3±0.4	15.8±0.3	137.5±14.2	1.1±0.2	9.9±2.9	41.7±1.2	4.7±0.3	10.9±1.9	57.3±1.1	82.7±18.0
	Olearia avicenniifolia	154±28	370.4±26.8	0.40±0.05	14.8±1.1	1.1±0.3	17.0±2.2	117.7±19.3	1.3±0.2	10.0±2.5	14.2±0.7	10.6±1.7	5.5±0.9	30.2±1.1	67.2±21.6
2	*Aristotelia serrata*	83±4	242.5±10.3	0.34±0.01	23.1±0.4	2.5±0.1	11.3±0.8	139.2±13.8	1.8±0.1	22.1±2.3	83.3±3.4	41.3±3.7	6.2±1.0	130.7±4.4	83.3±13.4
(65y)	*Coriaria arborea*	119±5	324.7±9.9	0.37±0.01	20.4±0.2	1.3±0.1	7.2±1.4	61.7±12.6	1.6±0.1	14.0±1.4	70.1±14.4	13.2±0.6	9.1±1.4	116.4±8.7	60.2±12.7
	Coprosma lucida	95±17	315.6±25.9	0.29±0.04	17.2±2.9	1.1±0.2	7.6±1.0	84.7±10.4	0.7±0.2	7.1±0.8	51.9±14.0	31.7±4.3	4.3±0.9	88.0±16.2	20.4±5.1
	Melicytus ramiflorus	64±10	238.1±9.6	0.27±0.04	26.0±2.6	2.5±0.5	6.1±1.0	94.9±11.3	1.0±0.1	16.8±2.4	46.7±4.3	31.7±6.3	8.6±2.0	87.1±11.2	27.1±10.7
	Olearia arborescens	80±3	282.4±9.8	0.28±0.00	17.7±1.8	1.7±0.2	12.5±0.9	158.4±15.6	0.9±0.1	12.1±1.5	17.1±2.9	5.6±0.5	4.6±0.8	27.2±3.5	36.4±2.4
	Schefflera digitata	69±6	211.4±11.7	0.33±0.01	21.6±1.0	1.7±0.1	6.1±0.9	91.1±15.5	1.0±0.1	15.6±1.8	36.1±1.5	32.1±3.5	3.3±1.8	71.6±3.0	91.6±15.0
3	*Fuchsia excorticata*	50±4	209.9±19.1	0.25±0.03	22.0±1.9	2.3±0.2	17.8±3.0	369.4±72.1	1.9±0.3	40.6±9.4	83.4±6.8	1.0±0.4	0±0.7	84.3±7.2	15.5±0.20
(135y)	*Griselinia littoralis*	236±13	643.1±32.5	0.37±0.01	7.5±1.4	0.8±0.1	7.4±0.9	31.6±2.8	0.8±0.2	3.2±0.4	44.6±3.5	18.6±1.8	3.0±0.23	66.3±4.9	71.9±12.7
	Metrosideros umbellata	231±12	474.6±22.1	0.49±0.01	8.7±0.1	0.8±0.1	2.2±0.5	10.5±4.5	1.1±0.2	4.4±0.8	30.8±2.5	7.7±2.2	6.5±0.5	45.0±4.3	54.3±2.5
	Olearia illicifolia	111±17	335.4±14.1	0.33±0.04	21.0±2.8	2.3±0.5	24.5±4.4	249.2±65.6	2.7±0.4	25.6±4.4	22.5±2.7	15.8±4.6	6.0±1.3	44.6±8.0	15.3±2.7
	Pseudopanax colensoi	271±16	608.9±38.2	0.44±0.01	9.1±0.7	1.0±0.2	6.0±1.7	21.1±5.1	1.1±0.2	3.9±0.6	32.8±1.6	18.1±1.9	7.0±0.8	57.9±3.4	44.4±10.6
	P. crassifolius	280±31	602.1±44.7	0.46±0.02	8.9±0.9	0.6±0.1	10.2±3.4	39.7±15.6	1.0±0.2	3.8±1.0	25.7±2.8	12.5±1.1	1.6±0.5	39.8±2.6	30.1±6.9
	Weinmannia racemosa	230±14	466.9±24.5	0.49±0.01	10.1±0.7	0.8±0.1	4.7±1.0	20.2±3.9	1.2±0.2	5.1±0.5	75.7±3.6	3.9±0.71	0±0.5	78.3±4.3	76.0±4.1
4	*Griselinia littoralis*	162±25	460.5±53.7	0.35±0.01	9.7±1.1	0.9±0.1	3.7±1.3	17.3±5.0	1.2±0.5	5.2±2.4	51.1±3.5	21.7±4.2	6.0±2.6	78.8±9.7	67.0±14.4
(500y)	*M. umbellata*	245±10	476.7±19.7	0.51±0.00	9.4±0.6	0.7±0.1	7.8±1.8	33.6±8.3	1.1±0.3	4.4±1.0	43.7±1.7	11.4±1.0	4.1±0.8	59.2±2.5	26.1±1.0
	Weinmannia racemosa	231±23	477.0 ±36.9	0.48±0.01	10.0 ±0.6	0.9±0.2	1.9±0.3	8.4±1.6	0.7±0.1	3.1±0.3	76.7±0.9	6.2±0.4	0±1.2	82.8±1.3	71.0±7.7
5	*Dacrydium cupressinum*	294±40	595.6±82.1	0.50±0.02	8.8±0.4	0.5±0.1	1.7±0.8	6.0±2.6	0.5±0.1	1.9±0.4	18.7±0.5	5.8±0.7	6.2±0.6	30.7±0.5	67.7±7.8
(12ky)	*M. umbellata*	199±14	410.8±14.9	0.48±0.02	7.9±0.2	0.4±0.1	2.9±0.6	13.1±3.5	0.8±0.1	3.8±0.3	35.0±0.9	7.1±0.5	2.3±0.4	44.4±1.3	65.7±20.2
	Weinmannia racemosa	189±12	394.6±17.9	0.48±0.01	7.8±0.3	0.4±0.1	na	na	na	1.5±0.1	79.3±4.1	10.5±0.5	4.4±0.3	94.2±4.5	94.0±17.0
6	*D. cupressinum*	480±10	922.5±35.8	0.52±0.01	7.7±0.4	0.3±0.1	2.1±0.2	4.4±0.4	1.1±0.4	2.3±0.7	18.1±1.0	5.7±0.3	4.8±0.3	28.7±1.0	59.3±9.4
(120ky)	*M. umbellata*	248±7	501.9±6.7	0.49±0.01	7.5±0.6	0.4±0.1	5.5±1.1	22.6±4.5	1.4±0.4	5.7±1.8	40.3±2.1	7.1±0.4	3.0±0.4	50.3±2.4	43.9±10.2
	Weinmannia racemosa	219±14	466.8±24.3	0.47±0.01	8.2±0.5	0.3±0.1	1.1±0.3	6.5±1.4	0.8±0.1	2.8±0.7	89.5±4.4	9.6±0.9	3.8±0.6	102.9±5.4	60.9±4.8

Table S2. Pearson's correlation coefficients and probability table.

Correlation Table of Raw data pvalues/correlation	N	P	N_P	Gluc	Fruc	Suc	totSug	Starch	TNC	LMA	DMC	FMA	Rdark	A1500M	RdarkM	A1500N	RdarkN	A1500P	RdarkP
N		0.885	-0.324	0.260	0.431	0.315	0.418	-0.124	0.179	-0.764	-0.793	-0.715	0.360	0.604	0.706	0.415	0.458	0.311	0.227
P	0.000		-0.582	0.192	0.413	0.166	0.334	-0.118	0.131	-0.695	-0.792	-0.618	0.424	0.638	0.749	0.471	0.548	0.216	0.132
N_P	0.000	0.000		-0.058	-0.263	0.084	-0.134	-0.001	-0.085	0.363	0.430	0.274	-0.301	-0.323	-0.360	-0.314	-0.353	0.063	0.185
Gluc	0.003	0.027	0.509		0.198	-0.054	0.860	0.136	0.688	-0.312	-0.137	-0.349	0.005	0.089	0.209	-0.019	0.136	-0.091	0.066
Fruc	0.000	0.000	0.002	0.022		0.219	0.541	0.092	0.416	-0.388	-0.435	-0.321	0.103	0.029	0.210	-0.032	0.099	-0.061	-0.040
Suc	0.000	0.057	0.339	0.540	0.011		0.150	-0.044	0.074	-0.169	-0.197	-0.128	0.074	-0.030	0.023	-0.089	-0.077	0.046	-0.021
totSug	0.000	0.000	0.125	0.000	0.000	0.085		0.186	0.670	-0.437	-0.306	-0.437	0.078	0.068	0.263	-0.039	0.142	-0.095	0.039
Starch	0.157	0.180	0.994	0.118	0.291	0.616	0.032		0.734	0.033	0.149	-0.004	-0.145	-0.218	-0.163	-0.241	-0.194	-0.250	-0.178
TNC	0.040	0.134	0.331	0.000	0.000	0.396	0.000	0.000		-0.233	-0.078	-0.262	-0.085	-0.087	0.043	-0.188	-0.041	-0.229	-0.095
LMA	0.000	0.000	0.000	0.000	0.000	0.057	0.000	0.708	0.008		0.795	0.959	-0.236	-0.598	-0.626	-0.508	-0.492	-0.413	-0.258
DMC	0.000	0.000	0.000	0.122	0.000	0.026	0.000	0.094	0.382	0.000		0.627	-0.275	-0.644	-0.689	-0.537	-0.525	-0.376	-0.209
FMA	0.000	0.000	0.002	0.000	0.000	0.151	0.000	0.960	0.003	0.000	0.000		-0.217	-0.561	-0.588	-0.468	-0.468	-0.398	-0.292
A1500	0.000	0.000	0.002	0.172	0.844	0.428	0.257	0.008	0.022	0.000	0.000	0.000	0.588	0.815	0.589	0.888	0.638	0.829	0.459
Rdark	0.000	0.000	0.001	0.959	0.262	0.423	0.395	0.112	0.355	0.010	0.002	0.017		0.527	0.696	0.519	0.806	0.397	0.728
A1500M	0.000	0.000	0.000	0.341	0.758	0.746	0.467	0.018	0.349	0.000	0.000	0.000	0.000		0.843	0.949	0.801	0.760	0.518
RdarkM	0.000	0.000	0.000	0.023	0.022	0.805	0.004	0.077	0.643	0.000	0.000	0.000	0.000	0.000		0.719	0.914	0.504	0.622
A1500N	0.000	0.000	0.001	0.842	0.735	0.340	0.675	0.009	0.044	0.000	0.000	0.000	0.000	0.000	0.000		0.776	0.846	0.532
RdarkN	0.000	0.000	0.000	0.144	0.286	0.407	0.127	0.036	0.657	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.568	0.783
A1500P	0.001	0.020	0.501	0.330	0.512	0.623	0.311	0.007	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.580
RdarkP	0.014	0.155	0.046	0.477	0.666	0.820	0.673	0.055	0.308	0.005	0.024	0.001	0.000	0.000	0.000	0.000	0.000	0.000	

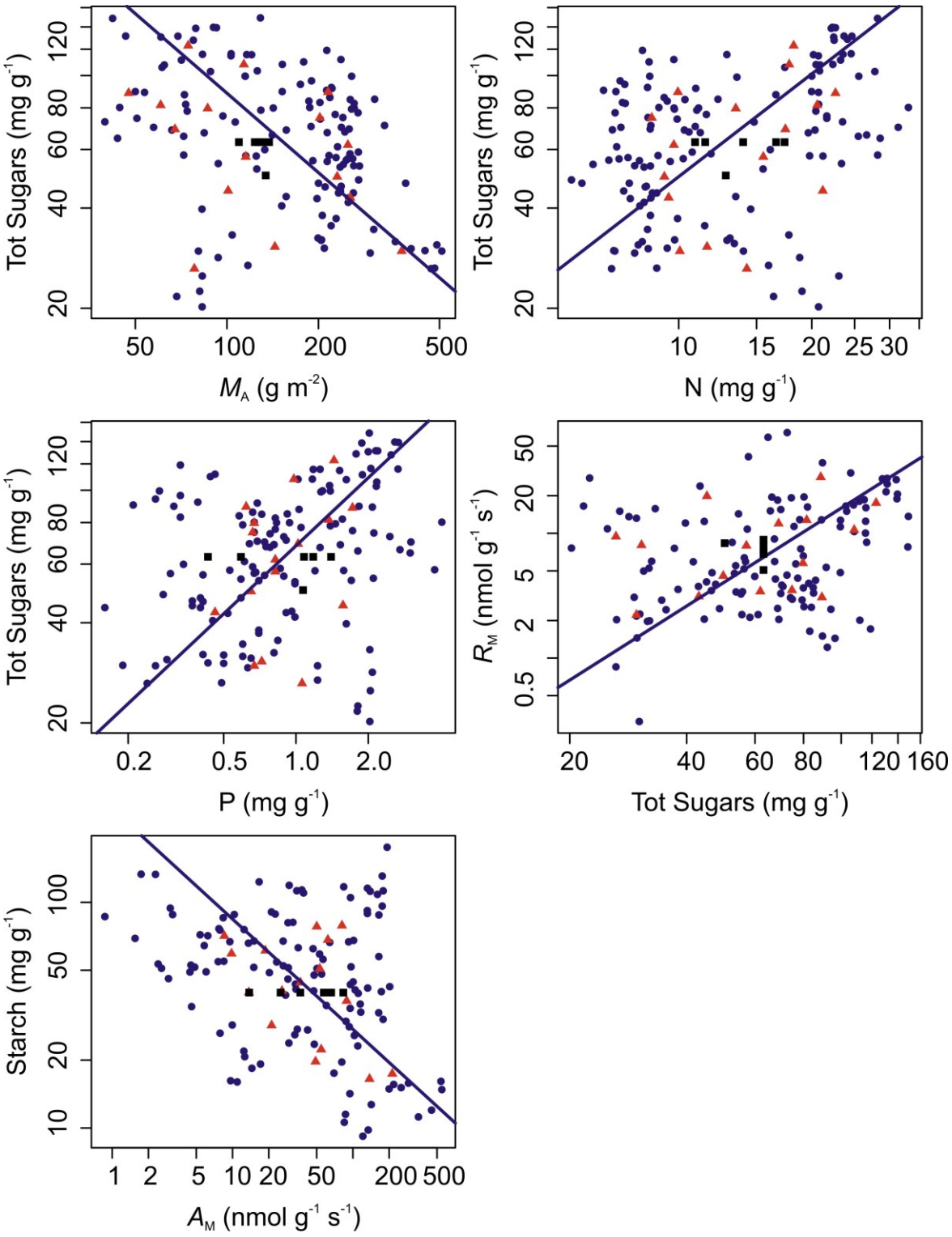

Figure S1. Log-log plots of area- and mass-based leaf gas exchange traits in relation to leaf mass per unit leaf area (M_A), foliar N and foliar P concentrations. Relationships shown for the complete data set (blue symbols), the genetically-driven (G) response (i.e. with the E component removed; red symbols) and for site averages (black squares). For these traits there was no difference between the complete and G-only relationships, so a single line is drawn. Details as for Figure 5.