1   Relationships between leaf mass per area and nutrient concentrations
2   in 98 Mediterranean woody species are determined by phylogeny,
3   habitat and leaf habit

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17

18   Abstract

19 Leaf structural and nutrient traits are key attributes of plant ecological strategies, as

20 these traits are related to resource use strategies and plant growth. However, leaf

21 structure and nutrient composition can vary among different habitats, leaf habits or

22 phylogenetic groups. In this study, we measured 13 leaf traits (one structural – leaf mass

23 per area, LMA – and 12 nutrient traits) in 98 Mediterranean woody species growing

24 over a wide range of environmental conditions, with the final aim of discerning the

25 main causes of leaf trait variability. The variance decomposition results show that

26 phylogeny, leaf habit and forest habitat affected in several ways the structural and

27 nutrient traits studied. Leaf nutrient concentrations are strongly positively correlated

28 amongst themselves, **and negatively correlated with LMA, in accordance with the "leaf**

29 **economics spectrum"**. We found that leaf habit and phylogeny were important causes of

30 variation in LMA and in a broad number of leaf nutrients (i.e. C, N, Mg, S, K), while

31 other micronutrients seemed to be more dependent on the environment (i.e. Cu and

32 Mn). In summary, our study reinforces the existence of the leaf economics spectrum in a

broad pool of Mediterranean woody species, and demonstrates the strong influence of phylogeny, leaf habit and environmental context as the main drivers of variability in some leaf structural and nutrient traits.

Keywords: Functional traits, Leaf economics spectrum, Nitrogen, Phosphorus, Phylogenetic independent contrast (PIC), Stoichiometry.

Key message: This study reinforces the existence of the *leaf economics spectrum* in Mediterranean woody species, and demonstrates the strong influence of phylogeny, leaf habit and environmental context as main drivers of variability in structural and nutrient traits of leaves.

Author contribution statement

EGdR, TM and RV conceived the ideas; EGdR, JLQ, IMP-R and LM collected the data; EGdR analysed the data; and all authors contributed to the writing.

Conflict of interest

Introduction

The leaf is the main organ for photosynthesis in higher plants. Consequently, leaf traits are closely related to the resource uptake and growth potential of plants (Niinemets and Kull 2003; Wright et al. 2004). Among leaf traits, Leaf Mass per Area (LMA) – or its inverse, specific leaf area (SLA) – is a structural trait widely used as an indicator of differential functional strategies in plant species (Wright et al. 2004; Díaz et al. 2016). Plant growth requires at least 17 mineral elements (Watanabe et al. 2007) that are taken from the soil (Brouwer, 1962), and are used in their leaves to support fundamental physiological processes (Asner et al. 2014). As a result, LMA and leaf nutrient concentration are often closely related across species, both in Mediterranean environments (Dominguez et al. 2012; Grubb et al. 2015; de la Riva et al. 2017) and

other biomes (Reich et al. 1999; Niinemets and Kull 2003; Wright et al. 2004; Chen et al. 2011), suggesting the existence of a global spectrum of leaf trait coordination and trade-offs.

The leaf economics spectrum theory (*LES*, Wright et al. 2004) indicates the existence of a trade-off between investment in mechanical support on one hand and investment in physiological activity on the other (Pratt et al. 2007). For instance, plant species with soft or thin leaf tissues (i.e., low LMA) and short-lived leaves tend to have a higher leaf N concentration, which potentially confers higher photosynthetic capacity and high growth rates (Poorter and Remkes 1990; Wright et al. 2004; Poorter and Bongers 2006; de la Riva et al. 2016b). On the contrary, plant species with higher LMA values have thicker and denser leaves and a greater proportion of C in structural tissues (i.e. vascular and sclerenchyma). In combination this enhances the protection of leaves against biophysical hazards, thus enhancing leaf lifespan and the duration of photosynthetic revenues from leaves. Other macro-or micronutrients such as Ca, Mg, S, Fe, Mn and Zn are also important but their relationships with LMA have been studied less (but see Niinemets and Kull 2003 and Fyllas et al. 2009).

The structural and nutrient concentration of leaves may be regulated by evolutionary and physiological processes (Reich et al. 1992 and 1999). For instance, a variety of nutrient uptake and use strategies may have facilitated the radiation and diversification of deciduous species during the Cretaceous (Axelrod, 1966), which promotes species coexistence by diverging in nutrient requirements according with their leaf habits (de la Riva et al. 2017). In addition, environmental conditions and habitat specialization can also strongly influence leaf nutrient concentrations (Ågren 2008; Sardans et al. 2015). Thus, nutrient uptake and utilization may reflect a balance between nutrient availability in the environment and the basic metabolic requirements of plants (Sterner and Elser, 2002), where micronutrient concentrations seem to be more determined by the availability in the environment and luxury uptake whereas macronutrient concentrations seem to be more determined by plant requirements (Hans et al. 2011; Zhao et al. 2016). Leaf structure, nutrient composition and their relationships may vary therefore with habitat conditions, leaf habit or evolutionary history (Niinemets and Kull 2003; McGroddy et al. 2004; Watanabe et al. 2007; Chen et al. 2011; Sardans et al. 2015; de la Riva et al 2017). Yet, how much of the trait variation can be explained by each of these factors remains largely unknown (Asner et al. 2014).

94   Knowing how leaf nutrient concentrations are related (i.e., the stoichiometry) is

95   also very important for a better understanding of plant functioning. Stoichiometric

96   homoeostasis is defined as the ability to maintain a given elemental composition despite

97   fluctuations in the environment (Sterner and Elser 2002), and such homeostasis is very

98   relevant to plant fitness and species strategies (Yu et al. 2011). According to the

99   "*biogeochemical niche hypothesis*", different plant species show an elemental balance

100 that is "optimal" for functioning in their specific environmental niche (Peñuelas et al.

101 2008). Thus, the "optimal" stoichiometry would be the result of the evolutionary history

102 under specific environmental conditions, leading to a determined plant strategy, even

103 though some degree of plasticity exists because of local environmental conditions and

104 competitive interactions (Sardans et al. 2015).

105 Variation in C:N:P ratios has been most investigated, since N and P are

106 commonly considered the most limiting nutrients for plant growth (Elser et al. 2007).

107 However, contrasting predictions have been made; Tilman´s resource ratio hypothesis

108 (Tilman 1997) assumes that plants take up nutrients in the proportions needed for

109 growth, whereas Koerselman and Meuleman (1996) suggest that the ratios of nutrients

110 in plant biomass simply reflect the relative amounts of nutrients available to plants.

111 Indeed, contradictory patterns for these nutrient ratios have been found (i.e. Niinemets

112 and Kull 2003). Because N is often the most limiting nutrient for growth (Vitousek and

113 Howarth 1991), it frequently shows the lowest coefficient of variation (Marañon et al.

114 2015; Zhao et al. 2016). Thus, cross–species variation in the ratios between leaf N and

115 other nutrients reflects physiological scaling relationships in nutrient requirements

116 (Knecht and Göransson 2004), which is crucial to understand plant nutrition and

117 nutrient deficits (Güsewell 2004; Knecht and Göransson 2004). However, the ratios of

118 N to other macro or micronutrients – such as K, Mg, Ca or Fe – have been barely

119 explored (Urbina et al. 2015).

120 In this study, we analyse the relationships between leaf structure and leaf

121 nutrient concentrations in 98 Mediterranean woody species growing in five different

122 vegetation types (including forests and shrublands), within the Southern Iberian

123 Peninsula, that encompass a wide range of environmental conditions (mainly aridity).

124 We first explored the spectrum of variation of one of the most–relevant structural traits

125 (LMA) and of 12 leaf nutrients –including macronutrients (C, N, P, K, Ca, Mg and S)

126 and micronutrients (i.e. Mn, Zn, Fe, Cu, and B)–along this gradient of aridity (from sub–

humid forests to arid shrublands). We selected LMA as an anchor trait due to its well–known importance as a functional indicator of resource–use strategies (Wright et al. 2004; Poorter et al. 2009). We tried to discern the proximate causes of this variability in leaf traits. Specifically:

We hypothesize that phylogeny and leaf habit explain most of the variation in LMA and macronutrients due to the higher relationship of them with physiological processes; whereas environmental conditions explain most of the variation in micronutrients because micronutrients are mainly determined by their availability in the environment (Hans et al. 2011; Zhao et al. 2016).

Second, we explored the stoichiometry of N with the other leaf macro and micronutrients (i.e., the ratio between N and other nutrients) because N is quantitatively the most–important nutrient for plant growth (Güsewell 2004; Lambers 2006). In line with LES theory, we hypothesize a strong covariation between LMA and nutrients, with fast–growing species with low LMA having higher nutrient concentrations, as some studies have previously documented in Mediterranean environments (Domínguez et al. 2012; Grub et al. 2015; de la Riva et al. 2016a and 2017).

Third, we hypothesized that species adapted to different habitats will display different nutrient composition and stoichiometry ratios. Because, if N is the nutrient with most stable concentration among different sites or forest (Güsewell 2004; Hans et al. 2011), we expect that the stoichiometry balance of N will be mostly conditioned by the availability of the other nutrients in each habitat types.

Material and Methods

Data collection

We selected most abundant and representative woody species occurring in forest and shrublands of 13 sites distributed across South Spain (Table S1 available as Online Resource). They covered a wide range of environmental conditions, from high mountain forests in Sierra Nevada to coastal shrublands in Doñana, from sub–humid forest in Cadiz to arid shrublands in Almeria. The 13 study zones were then grouped into five broad habitat types, based on the European classification of natural habitats (European

Commission 2013). These five habitats are: 1) Shrubland "forest" (hereafter *SF*), which includes the arid and semi–arid shrublands, 2) Evergreen oak forest (hereafter *EOF*), which includes the sclerophyllous Mediterranean oak forests of *Quercus ilex* L. and *Q. suber* L., 3) Pine forest (hereafter *PIF*), which includes the *Pinus sylvestris* L. forest of mid–elevation mountains, 4) Deciduous oak forest (hereafter *DOF*), which includes the Mediterranean deciduous oak forests of *Quercus canariensis* Wild. and *Quercus faginea* Lam., and 5) Riparian forest (hereafter *RIF*), which includes the deciduous–tree forests (e.g. with dominant species such as *Fraxinus angustifolia* Vahl. and *Ulmus minor* Mill.) associated with permanent water availability.

In each study site we measured LMA and leaf nutrient composition of most abundant woody species, many of them occurring in more than one study area (a total of 172 observations and 98 species; Table S2, available as Online Resource). In spring, we selected six individuals per species and sampling site, collecting five or six fully–expanded leaves per individual. Leaf mass per area (LMA, leaf dry mass per unit of area, g m^{-2}) was measured according to the methods described by Pérez–Harguindeguy et al. (2013). Leaf nutrient concentrations were determined for a mixture of leaves from five or six different individuals, per species and sampling site, selected at random. The N and C concentrations were measured using an elemental analyser (Eurovector EA 3000, EuroVector SpA, Milan, Italy). The macronutrients P, K, S, Ca and Mg and the micronutrients B, Cu, Fe, Mn and Zn were extracted by wet oxidation with concentrated HNO_3, under pressure in a microwave digester, and analysed by ICP–OES. The quotients between the leaf concentration of N and those of the other 11 nutrients (N:nutrients) were calculated on a mass basis.

The species were sorted into four leaf habit categories: deciduous (hereafter *De*), summer semi–deciduous (hereafter *Sd*), evergreen (hereafter *Ev*) and evergreen needles (hereafter *Ne*). To obtain a reasonable sample size for phylogenetic groups, the species were separated in ten groups attending to their evolutionary distance (around 100 million years; see Fig. S1, available as Online Resource). The species from the Division Magnoliophyta [*Berberis hispanica* (Boiss. & Reut.) Malag., *Osyris alba* L., *Ruscus aculeatus* L. and *Smylax aspera* L.] were grouped together, and *Ephedra fragilis* Desf. was grouped with the closest group (gymnosperms). *Vitis vinifera* L. and *Armeria vetulina* Boiss. & Reut. were discarded for this analysis because they could not be grouped with any related phylogenetic group (see Table S3 for details).

190 Data analyses

191 A principal component analysis (PCA) was performed with the complete set of 13 leaf
192 variables (LMA and 12 nutrients) for the 172 observations to explore the main trends of
193 variation, and the distribution of leaf habit types and phylogenetic groups in the
194 multivariate space (e.g. Reich et al., 1999, Fyllas et al. 2009, Sardans et al. 2015). To
195 assess the influence of the phylogeny, habitat type and leaf habit, as fixed factors, we
196 used linear mixed models with the PCA scores of the first and second components as
197 the dependent variables and the species as the random variable.

198 The variability of the 12 leaf nutrient traits was measured as the coefficient of
199 variation (CV; e.g. Zhao et al. 2016).

200 To assess separately the influence of each explanatory factor on each of the
201 studied leaf traits (LMA and 12 nutrients), a variance component analysis was
202 performed. For this, we fitted a general linear model with a factorial design, using a
203 traditional Type I sum-of-squares (Gower 1963). This approach can be used to estimate
204 different group-level regression coefficients and their variation in unbalanced datasets
205 (Gelman and Hill, 2006). Due to the unbalanced nature of our dataset, we verified the
206 robustness of our results with linear mixed models (species as random effect) for the
207 explanatory factors, which explained at least 10 % of the variance. In addition, *post hoc*
208 Tukey tests were performed to check the significance of differences between the least
209 square means of each group within each factor.

210 The relationship between LMA and leaf nutrient traits was analysed using a
211 linear mixed model, considering leaf traits as the fixed variables and species as the
212 random variable. The correlation of these relationships (r) was obtained with the
213 marginal R^2, which was calculated with the sem.model.fits function in the
214 'piecewiseSEM' package (Lefcheck 2015). To assess whether the relationships between
215 LMA and nutrient traits are also consistent within environments located at different
216 regional positions, the same analyses were performed within each habitat independently.
217 We did not test the relationships between LMA and nutrients or N: nutrients for each
218 phylogenetic group separately, since there were not enough species (n < 9 in 6 of the 10
219 groups) for statistical consistency.

7

220   To allow for the influence of species evolutionary history, the above-described
221 relationships were also determined by fitting a phylogenetic generalised least squares
222 (pgls) model. First, we calculated the average value for each species (a total of 98
223 woody species). Second, we calculated the phylogenetically independent contrasts
224 (PICs), which can assess the impact of phylogeny on our results (Webb et al. 2008;
225 Verdú and Pausas 2013). For these PICs, we used the pgls function of the caper package
226 for R (R Foundation for Statistical Computing, Vienna, AT), which addresses
227 phylogenetic non-independence among species by incorporating covariance between
228 taxa into the calculation of the estimated coefficients. For more specifications related to
229 the building of the phylogenetic tree, see de la Riva et al. (2016b).

230   To allow us to compare the stoichiometry results appropriately (based on the
231 slopes) with other studies (e.g. with the "optimum nutrient ratios" proposed by Knecht
232 and Göransson 2004), we assessed the bivariate relationships between N and other
233 nutrients with Standardised Major Axis Regression Tests (SMART; Warton et al. 2006)
234 (for the 172 observations combined and for each independent forest habitat). But, in
235 order to ensure that these relationships are not the result of a determined group of
236 species, their significance was also determined by linear mixed models, considering the
237 species as the random factor. In addition, PIC was carried out to control the
238 phylogenetic constraints.

239   The variables were log-transformed, if necessary, to meet assumptions of
240 normality (based on the Kolmogorov–Smirnov test) and homoscedasticity (based on the
241 Levene test). The PCA and linear mixed model analyses were conducted in the R 2·10·0
242 statistical platform (R Development Core Team 2011), using the packages 'FactoMiner'
243 (Lê et al. 2008), 'smatr' (Warton et al. 2012) and 'nlme' (Pinheiro et al. 2015). The
244 variance component analysis was performed with Statistica 10.0 (Statsoft, Tulsa, OK,
245 USA).

246

Results

Trait associations: general trends

249 To evaluate how the leaf and nutrient traits were related we first carried out a PCA. The
250 first PCA axis (explaining 31.0 % of the total variance) showed a high loading of leaf

nutrients (N, K, Ca, Mg, B, S) to the left and a high loading of LMA and C to the right. The second PCA axis (which explained 11.9 % of the variance) exhibited a high loading of Zn and Cu at the top and of P at the bottom (Fig. 1A). The positions of the 98 species along the biplot of the first and second PCA axes reflect the phylogenetic groups (Fig. 1A) and the leaf habit types (Fig. 1B), while no significant differences were found among forest habitats (Fig. S2, available as Online Resource).

There was a separation among the ten phylogenetic groups along the first PCA axis (Fig. 1A). *Fabales* (group D) appeared on the left side (with higher leaf nutrient concentrations), while *Gymnosperms* (J) and *Fagales* (B) were on the right side (with the opposite trait values) and the other groups had intermediate scores. There were also differences corresponding to leaf habit. Species with deciduous leaves and, to a lesser extent, with semi–deciduous leaves were separated along the first PCA axis (with higher leaf levels of N, Ca and Mg) from species with evergreen or needle leaves (with higher LMA values; Fig. 1B). However, the second PCA axis separated the species with deciduous leaves, which were richer in P, from the semi–deciduous species, which were poorer in P and had higher concentrations of some metals (Zn, Cu and Fe). With respect to the habitats, despite the absence of significant differences among the PCA scores, species from *RIP* tended to occur towards the negative extreme (P–richer) of the second axis, while those of the other three forest types (*DOF*, *SF* and *EOF*) were towards the positive end (Fig. S2, available as Online Resource).

Influence of phylogeny, forest habitat and leaf habit on trait variability

The coefficient of variation of the leaf nutrient concentrations across the 172 plant leaves analysed ranged from 6.7 % for C to 160 % for Cu (Fig. 2), with significant differences (t–test, $P<0.01$) among the micronutrients (111.4 %) and macronutrients (45.9 %). Variance partitioning analysis indicated that phylogeny caused most of the variation in N, Ca, K, Mn and Mg, whereas forest habitat caused most of the variation in P, Cu and S. Variation in leaf habit explained >20 % of the variation in LMA, N, Ca, Fe, Zn and Mg (Fig. 3). Only 7 % of the variation in C (not shown) could be explained by these three factors.

281   The *Liliopsida*, *Santalales* and *Ranunculales* (group J) had the highest nutrient

282   concentrations, whereas *Malvales*, *Violales* and *Sapindales* (group A) and *Ericales*

283   (group F) had the lowest values. In terms of leaf habit, (semi)–deciduous species had the

284   highest values for N, B, Ca and Mg (which were also related with *Rosales* and *Fabales*),

285   whereas evergreen species and, especially, needle–leaved species had higher LMA and

286   Fe. Species from *DOF* had the highest Cu and lowest P concentrations (see Fig. 3).

287

288   *Relationships between LMA and leaf nutrients*

289   The LMA was related significantly and positively with leaf C and negatively with the

290   concentration of most leaf nutrients (Table 1), except for Fe, Mn and Zn. All the

291   significant relationships observed were consistent after considering PICs (Table S3,

292   available as Online Resource). However, when analysing separately by habitat types,

293   many of the relationships among the LMA and nutrient traits were not significant. Only

294   the group of species from the pine forest maintained the significant relationships (except

295   for C). Among the variables, the relationship LMA–Mg was significant for four of the

296   five forest habitats (Table 1). The LMA was related significantly and positively with

297   C:N (Fig. 4). Also, significant and positive relationships were found between LMA and

298   N:Mg ($P = 0.001$), as well as with N:Ca and N:Mn ($P = 0.04$ and $P = 0.01$,

299   respectively), but these two were phylogenetically dependent (Table S3).

300

301   *Stoichiometry*

302   The N concentration was related significantly and positively to all other nutrients except

303   Mn and Zn, when the whole species pool was considered (Table 2 and Fig. 5). These

304   relationships were generally also significant when using PICs (Table S3, available as

305   Online Resource). In general, the slopes were positive and greater than 1, indicating

306   increased levels of nutrients relative to N (except for P and C) (Table 2). For N and P,

307   the slope was less than 1 (0.83), indicating that when N increases, the P concentration

308   also increases but at a lower rate. However, for C the slope was negative (–4.6),

309   indicating a decrease in C when N increases. These general stoichiometric patterns

310   varied according to the forest habitat, but many of the relationships between N and the

311   other leaf nutrients were not significant, probably because of a smaller sample size and

a narrower range of trait values considered. *PIF* was the group with the most significant relationships (Table S4, available as Online Resource). Moreover, we found strong differences between groups, but without a regular pattern (Table S5, available as Online Resource).

Discussion

Variation in LMA and nutrient composition among woody species

Our analyses of variance partitioning for the leaf nutrients considered in this study revealed fairly unbalanced distributions across phylogeny, leaf habit or habitat type. Interestingly, these results were highly nutrient-dependent, which suggests different processes of nutrient regulation. Thus, while some nutrients seemed to be more related to evolutionary constraints (e.g., Mn, K, N, Mg or Ca) and leaf habit (B, Ca, Mg or N), for others the variation was mainly driven by environmental conditions (e.g., P, Cu, S or Fe). Thus, our results indicate that leaf nutrient concentration is determined by species constraints and evolutionary processes together with acclimation to the environmental conditions (Pärtel et al. 2007). Nevertheless, a high percentage of the variance (58 %, on average) was not explained by the three factors considered in our study (similar to Fyllas et al. 2009), likely due to the involvement of other potential factors associated to differences among sites, species or individuals (Watanabe et al. 2007; Messier et al. 2010; Auger and Shipley 2013)

As we hypothesized, leaf macronutrients were less variable than micronutrients (Zhao et al. 2016). Comparable results were obtained by Han et al. (2011) and Marañón et al. (2015), with lower variability for N, K and P, the three most-frequently-limiting nutrients, while trace elements such as Mn and Cu showed the highest variability. The physiological reasons have yet to be further elucidated (Zhao et al. 2016). According to the "*hypothesis of stability of limiting elements*" (Han et al., 2011), those elements that are largely necessary for the plant (frequently scarce) are often limited by stoichiometric requirements. Hence, the extreme lower values of these elements could be non-viable from a physiological point of view, while the higher values could imply higher growth rates, diluting the increase of the nutrient with the increase of the mass.

342   The general pattern of LMA and leaf nutrients followed the acquisition–
343 conservation trade–off (Wright et al. 2004). In this respect, leaf habit and evolutionary
344 context are important leaf–history attributes of plants in the determination of LMA and
345 leaf nutrient concentrations (Reich et al. 1992; Sardans et al. 2015), and some links
346 among these two types of attribute exist. Thus, the deciduous species (commonly
347 associated with acquisitive strategies) and the semi–deciduous species from the *Fabales*
348 group (N fixers; Cleveland et al. 1999) showed higher nutrient concentrations and lower
349 values of LMA. In contrast, the evergreen angiosperms, especially from *Malvales*,
350 *Violales*, *Sapindales*, *Ericales* and *Fagales*, and the needle–bearing gymnosperms
351 showed opposite syndromes (related with a conservative resource–use strategy; cf. de la
352 Riva et al. 2016a, b), which favours slow growth rates and more–efficient use of
353 nutrients, resulting in low nutrient requirements (Ryser 1996). It is of note that the semi–
354 deciduous species showed some patterns similar to those of the deciduous species (Fig.
355 1 and Fig. 3), despite their contrasting habitats. The semi–deciduous habit is typical of
356 dry Mediterranean conditions, in which shrubs are able to shed partly or completely
357 their leaves during summer to reduce water loss by transpiration (Zunzunegui et al.
358 2005; Ciccarelli et al. 2016). Plant functional convergence often occurs as an adaptation
359 to similar environmental stresses, which promote similar functional and physiological
360 traits (see Jacobsen et al. 2008). In our case, winter deciduous and summer semi–
361 deciduous species displayed similar patterns regarding leaf nutrients (especially Ca, Mg,
362 B and N). These results suggest similar functional physiological solutions to different
363 environmental stresses. In both cases, the leaf life–spans are shorter, which is related to
364 high relative nutrient requirement and low resistance to physical hazards (Ryser 1996).
365 Therefore, our results support the existence of different nutrient–use strategies
366 associated with differences in leaf habit, which could result in different functional
367 adaptive solutions, constraining the evolutionary processes (Sardans et al. 2015).

368   Nutrients more related to the environment were not clearly aligned with this
369 nutrient–uptake strategy. Thus, habitat type was a main factor determining the leaf
370 concentrations of some heavy metals (Fe, Zn, Cu), as well as of P and S, supporting that
371 environmental conditions usually act as important drivers of nutrient concentrations in
372 terrestrial plants (Asner et al. 2014; Zhao et al. 2016). The strong influence of habitat
373 type on leaf concentrations of trace elements may arise because they are determined
374 largely by anthropogenic sources in the environment and by the selective uptake of soil

elements, the differential exclusion or accumulation and the transport within plants (Broadley et al. 2007; Marañón et al. 2015). The Cu concentration was related almost exclusively with habitat type, showing also the highest CV. This result could be partly explained by the fact that one of our study sites (El Molino Canyon) showed high values of soil Cu (up to 34 mg kg^{-1}), probably due to its proximity to a vein rich in Cu (Navarro-Fernández et al. 2016). This result suggests that, although macronutrients are usually more mobile than micronutrients (i.e. trace metals) (Zhao et al. 2016), leaves can accumulate more metals than required for plant demand, depending on the soil conditions.

Leaf P was also strongly dependent on the environment (habitat type) and only weakly determined by phylogeny, supporting recent studies (Asner et al. 2014; Zhao et al. 2016). Leaf P decreases with higher temperature, lower precipitation, and higher water stress (Reich and Oleksyn 2004; Yuan and Chen 2009; Sardans et al. 2011), because increasing aridity reduces biological activity and limits the soil supply of P (Delgado-Baquerizo et al. 2013). This could also explain the low values of P found in the most-arid sites (shrublands), compared to the *RIF*. In addition, aridity limits plant size (de la Riva et al. 2016c), which seems to be related with P acquisition; shrubs tend to show lower values of leaf P than trees because of their lower capacity to maintain larger root systems, which would allow them to explore large soil volumes and access the immobile available P (see Niinemets and Kull 2003 and references therein). However, the symbiosis with mycorrhizal fungi might improve the ability of different woody species to take up P and should be also considered (Navarro-Fernández et al. 2016).

Correlates of the nutrient concentrations and LMA of woody species

Strong relationships between the LMA and the concentrations of some elements were found in the 98-woody species analysed in this study. Across the species, LMA was related negatively to most of the leaf nutrients studied, and these relationships were not affected when phylogenetic relatedness was considered, providing some evidence of convergent evolution between leaf structure and nutrient concentration. Our results agree with the general global relationship between LMA and N and P, broadly known as the "*Leaf Economic Spectrum*" (*LES*, Wright et al. 2004), which could be extended to

other nutrients less frequently analysed such as Ca, K, Mg, B, Cu or S. Thus, species with a higher LMA tend to have lower values of nutrients and low rates of photosynthesis and respiration, which takes longer to pay back the leaf construction costs (Villar and Merino 2001; Wright et al. 2004; Villar et al. 2006). In this respect, leaves with longer life–spans require greater mechanical support, so the increase in LMA is related to a greater proportion of C in structural tissue in woody plants (Villar et al. 2013; de la Riva et al. 2016a), which makes them less susceptible to environmental hazards (Poorter and Villar 1997; Poorter et al. 2009). By contrast, higher concentrations of leaf nutrients lead to higher photosynthetic rates per unit mass (in the case of N), greater control of stomatal opening (due to K) and higher rates of protein synthesis (Ca and Mg) (Mayland 1990; Egilla et al. 2005; Villar et al. 2006; Hashimoto and Kudla 2011). The LMA was positively related to the C:N ratio. This ratio gives an idea of the relative investment in structure (C) and cell functioning (N); species having leaves with a higher C:N ratio are usually slow–growing (Poorter and de Jong 1999; Villar et al. 2006).

However, the general relationships between leaf structure and nutrients became weaker, or disappeared, when considering groups of species with the same leaf habit or growing in environmentally–similar forest habitats, which may reflect an under–appreciated dimension of the *LES* (Fyllas et al. 2009). Our results, and previous studies based mainly on morphological leaf traits (Funk and Cornwell 2013; de la Riva et al. 2016b), suggest that these differences could be related to the spatial scale. Thus, for a broad range of species a strong relationship among structure and nutrients exists. But, when we consider groups of species growing in similar environments, which have been selected through the same filter, the range of trait variation is smaller, and the relationships could be weaker (Niinemets and Sack 2006). Hence, these relationships do not necessarily match the global pattern (Funk and Cornwell 2013).

Stoichiometric relationships and variations among habitats

There were positive relationships between N and the concentrations of most of the nutrients considered (except for C, which was related negatively), with significant differences between habitat types. Considering as reference values the "optimum nutrient ratios" (Knecht and Göransson 2004), Ca, Mg and K showed ratios higher than

the optimum, but the P ratio was slightly lower than expected (Fig. 5). This indicates that plants are mostly limited by either N or P, while the other elements can be taken up in excess of requirements for growth (Knecht and Göransson 2004). Thus, our results suggest that strong regulation exists for N and P, while other nutrients – such as K, Mg and Ca – show less or no regulation. The N:P ratio showed a slope of 0.83, indicating that P accumulates at a lower rate than N. This value is similar to those (0.77–0.93) obtained in tropical forests (McGroddy et al. 2004; Townsend et al. 2007; Fyllas et al. 2009), but somewhat higher than the overall average values quantified in temperate forests (McGroddy et al. 2004) and in the *LES* global database (0.66, Wright et al. 2004; Reich et al. 2010), suggesting that some P limitation exists in the environments of the present study (Domínguez et al. 2010).

Nevertheless, the results obtained for each habitat suggest that some degree of flexibility exists in these stoichiometric ratios. This variability among habitats may be due to the different ecological strategies of the species that compose them – for example, different growth and nutrient supply rates – which allow the species to respond successfully to the environmental constraints of each particular habitat (Knecht and Göransson 2004; Sardans and Peñuelas 2015; de la Riva et al. 2017). These variations of the slopes could be explained by considering that all elements can be taken up in excess of the requirements for growth (Knecht and Göransson 2004). At a small spatial scale, differences among species with regard to their uptake and variability in the availability of elements in the soil are important aspects that need to be investigated further (Ågren et al. 2008). This study highlights the importance of considering the habitat when determining the nutrient supply and leaf stoichiometry within a heterogeneous pool of Mediterranean woody species.

In spite of the broadly–constrained stoichiometric ratios in forests worldwide (McGroddy et al. 2004), we found differences among habitat types. According to Güsewell (2004), variations in N could be more important in determining the stoichiometric ratios in woody plants, such as N:P. However, the differences in stoichiometric balance among habitat types seem to be related more to other nutrients than to N. For example, the habitat types with low leaf P (*DOF* and *SF*) showed the highest values of N:P, as well as the highest N:nutrient ratios with Fe, Cu, Ca, S and Mn. Similarly, McGroddy et al. (2004) found differences in N:P among habitats and proposed that these variations were associated with changes in P rather than in N, which

could be explained by the fact that N is the most-constant nutrient (after C) across environments. Temperate forest soils appear uniformly N–poor (Jenny 1950), which promotes adaptive solutions such as symbiotic N fixation. These adaptations, the fact that N is often the most-limited nutrient, and the capacity of plants to take up small excess amounts of N under non–limiting conditions (Knecht and Göransson 2004) seem to show that the stoichiometry of N:nutrient ratios in these ecosystems is conditioned more by the other elements involved. This suggests that the relationships among nutrients could have arisen through a small number of ancient events, but also that habitat specialisation drives the nutrient balance (Fyllas et al. 2009).

Conclusions

Taken together, our results highlight four important points concerning the natural variability of LMA and leaf nutrients across a wide pool of Mediterranean woody species. First, the leaf-trait patterns of this set of 98 Mediterranean woody species supported the general existence of the "*leaf economic spectrum*" for LMA and a broad number of nutrients; however, some nutrients –such as Cu and Mn –seemed to be more environment-dependent. Second, the variability of the leaf nutrient concentrations depended on phylogeny, leaf habit and habitat in different proportions: some nutrients were more phylogenetically constrained (i.e., Mn and K), while others showed stronger associations with either the environmental conditions (i.e., P, Cu and S) or leaf habit (i.e., N, B, Zn and Ca). Third, significant relationships exist between N and most of the other nutrients, for the whole species pool, but there is considerable variation among forest habitats. Fourth, strong regulation exists between N and P uptake; P seems to be the most-limited nutrient for the studied species, while plants are able to take up profligately other macronutrients (i.e., Ca, Mg and K). In summary, our study reinforces the existence of the leaf economics spectrum in a broad pool of Mediterranean woody species, and highlights the necessity of expanding this concept to other nutrients less frequently analysed such as Ca, Mg, K or S. In addition, our results demonstrate the strong influence of phylogeny, leaf habit and environmental context as the main drivers of variability in some leaf structural and nutrient traits, and provide relevant information on leaf nutrient stoichiometry in Mediterranean woody plants from natural environments.

Acknowledgements

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744 Figure legends

745

746 Figure 1. Plots of the principal component analysis (PCA). The scores for the 13
747 variables (LMA and nutrients) used for the PCA are displayed with their vectors. The
748 symbols within the PCA plots correspond to the species groups, according to phylogeny
749 (A) or leaf life–span (B). The coloured lines indicate the mean of the scores of the main
750 (A) phylogeny along factor 1 and factor 2 of the PCA and (B) leaf life–habit, when
751 significant differences exist (LMM, $P < 0.05$). Different letters indicate significant
752 differences between groups (Tukey test, $P < 0.05$). Phylogeny groups: (A) *Malvales*,
753 *Violales*, *Sapindales*, (B) *Fagales*, (C) *Rosales*, (D) *Fabales*, (E) *Malpighiales*, (F)
754 *Ericales*, (G) *Dipsacales*, *Apiales*, *Asterales* (H) *Lamiales*, *Solanales*, Gentianales, (I)
755 *Liliopsida*, *Santalales*, *Ranunculales* (J) *Gimnospermae.*

756

757 Figure 2. Coefficient of variation (CV) of the leaf concentrations of macro and
758 micronutrients for 172 plants belonging to 98 woody species.

759 Figure 3. Variance components analysis of LMA and leaf nutrients. For the best
760 explaining factors (>10 %), average differences based on linear mixed models are
761 shown: (A) traits mostly explained by the three factors (phylogeny, leaf life–habit and
762 forest habitat); (B) traits mostly explained by two factors; and (C) traits mostly
763 explained by one factor. The sub–groups obtained with the *post hoc* Tukey test ($P <$
764 0.05) are marked with different letters in the bars. Phylogeny groups: (A) *Malvales*,
765 *Violales*, *Sapindales*, (B) *Fagales*, (C) *Rosales*, (D) *Fabales*, (E) *Malpighiales*, (F)
766 *Ericales*, (G) *Dipsacales*, *Apiales*, *Asterales* (H) *Lamiales*, *Solanales*, *Gentianales*, (I)
767 *Liliopsida*, *Santalales*, *Ranunculales* (J) *Gimnospermae.*

768 Figure 4. Relationship between the leaf mass area (LMA) and the leaf C:N ratio. The
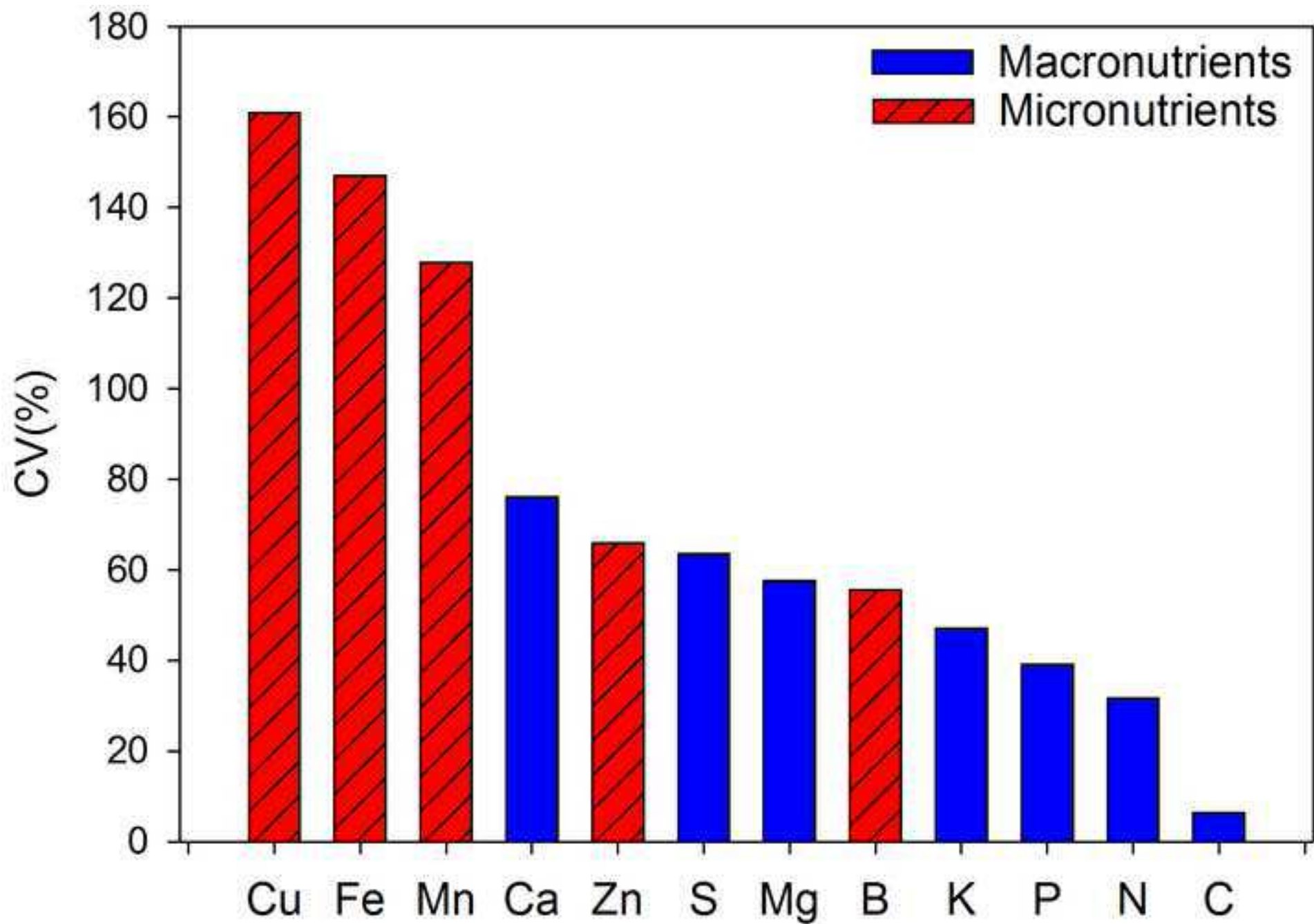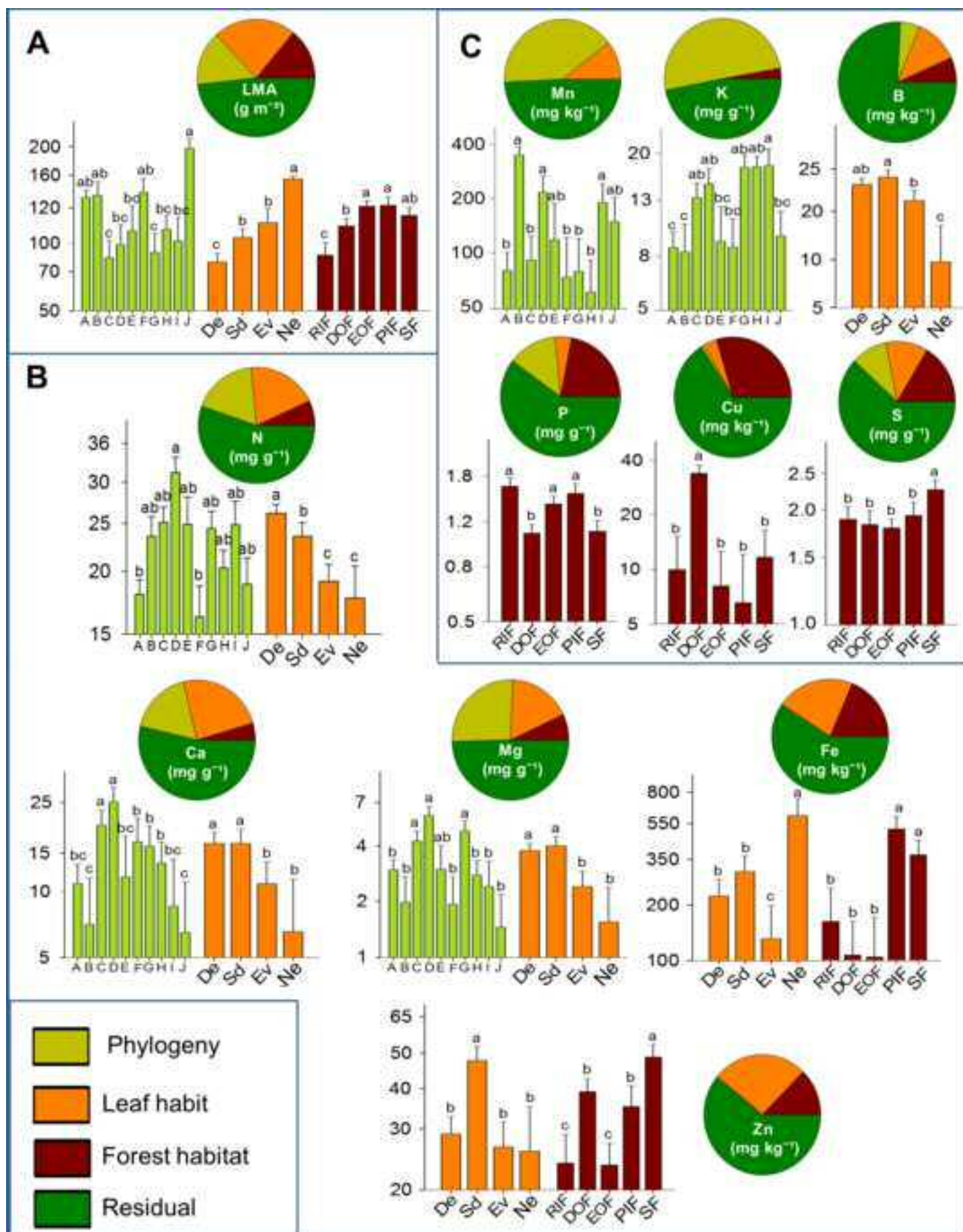769 marginal r and *P* values from the linear mixed model are also shown.

770

771 Figure 5. Standardised major axis (SMA) regressions of N versus the P, K, Ca and Mg
772 concentrations. The dashed lines represent the optimum nutrient ratio (based on Knecht
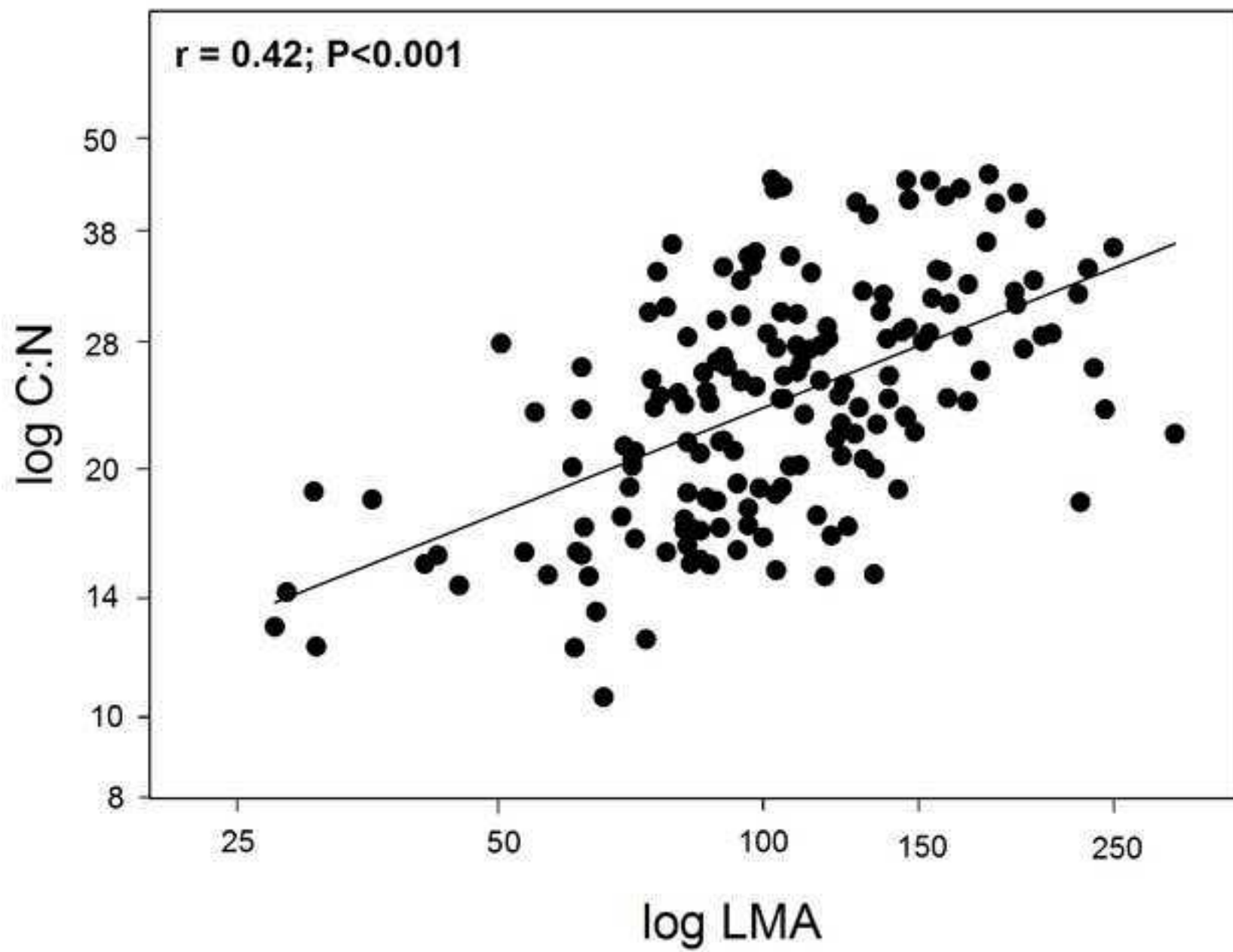773 and Göransson 2004).

774

775

A) Phylogeny

B) Leaf habit

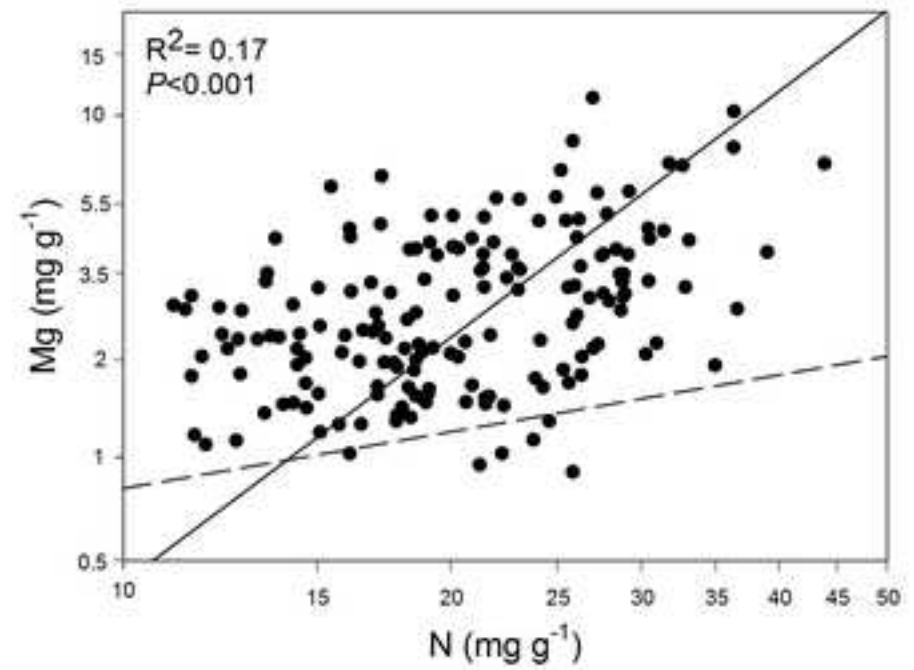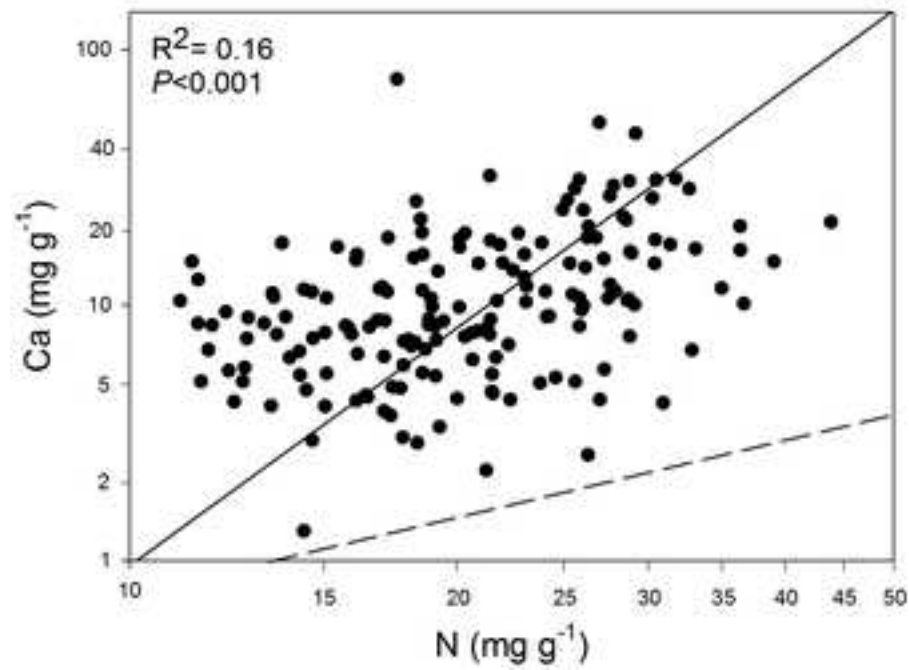Figure
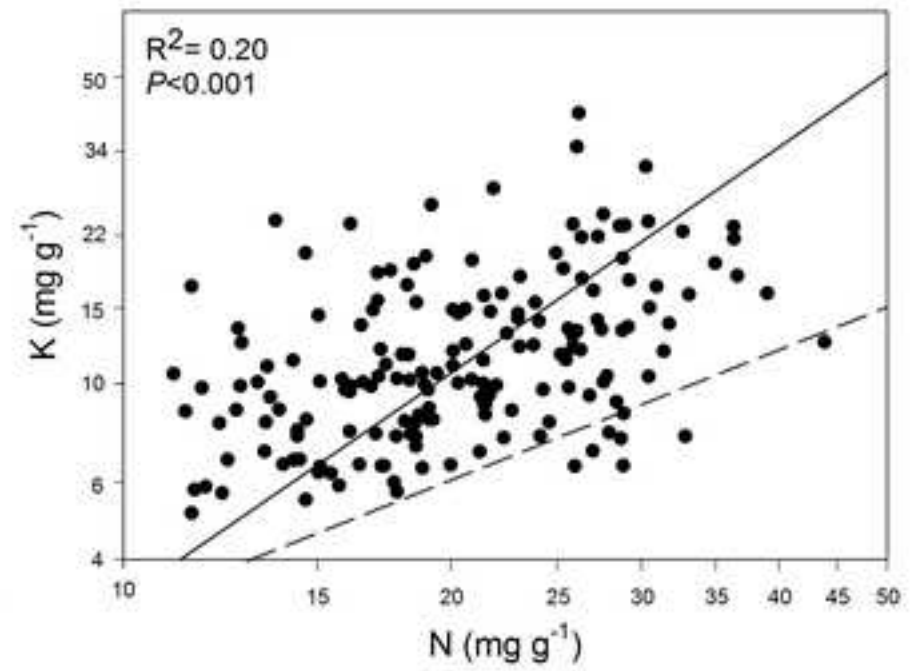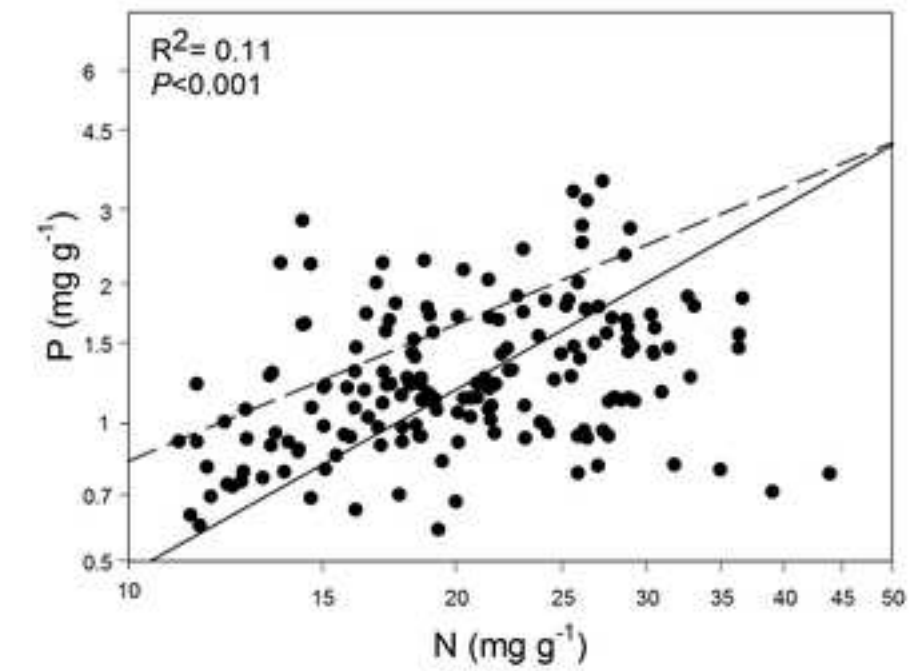
r = 0.42; P<0.001

log C:N

log LMA
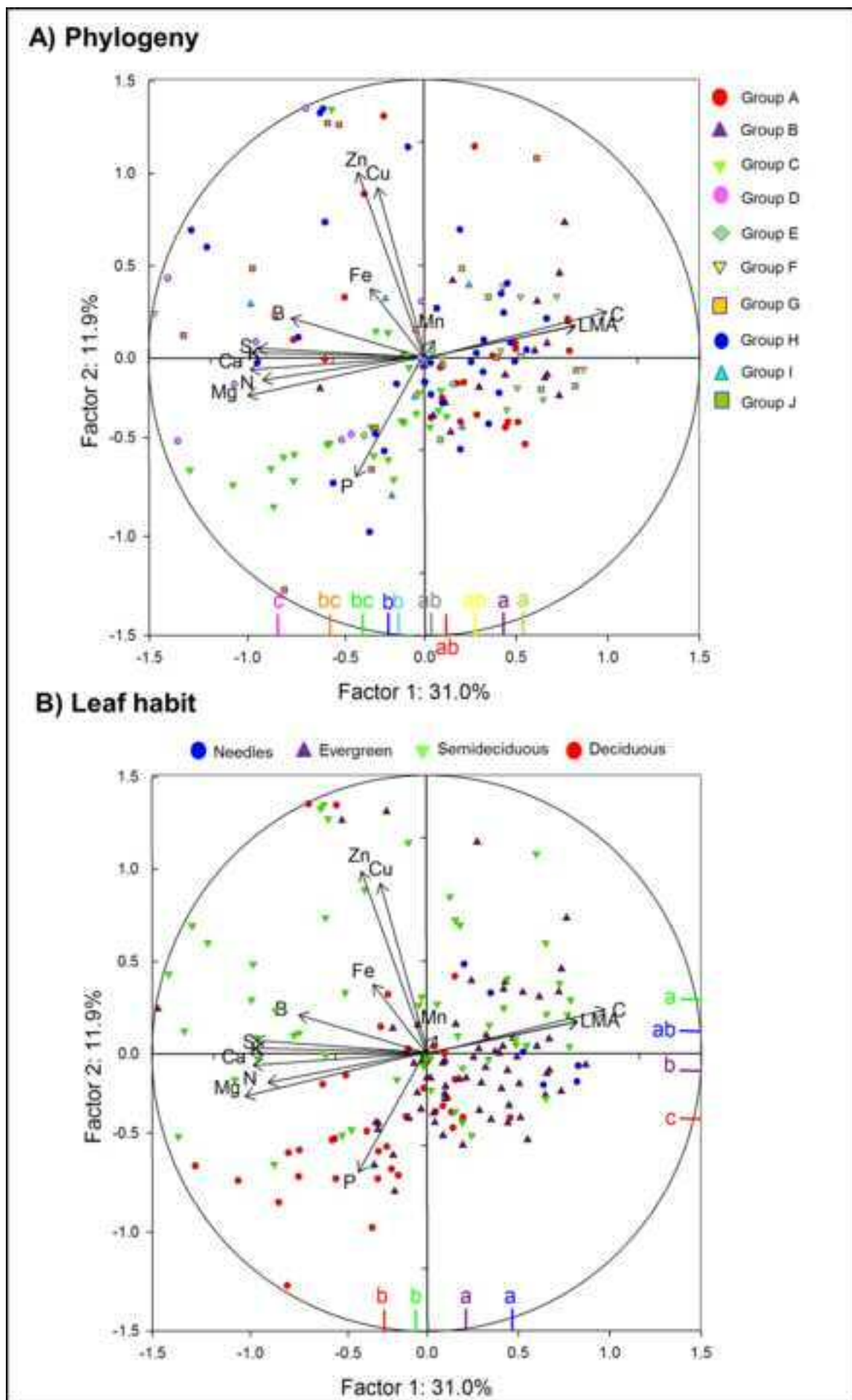
Table 1. Linear mixed model analyses of the relationships between leaf mass per area (LMA) and leaf nutrients, for the total pool and for the different habitats type. The marginal r and significance level are also shown (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (n) Indicates the number of cases and species used for each comparison. The data were $\log_{10}$-transformed prior to the analysis.

	Leaf nutrient	Total Pool	SF	EOF	PIF	DOF	RIF
		n (172,98)	n(41,36)	n(55,31)	n(21,21)	n(29,26)	n(26,14)
Macronutrients	[C]	**0.28***	0.12	0.12	0.31	**0.54***	**0.60****
	[N]	**-0.36***	**-0.40***	**-0.63***	**-0.68***	-0.20	**-0.66****
	[P]	**-0.34***	-0.05	-0.09	**-0.61****	-0.06	-0.24
	[Ca]	**-0.36***	**-0.49***	-0.03	**-0.64****	-0.34	-0.22
	[K]	**-0.42***	**-0.28***	**-0.27***	**-0.71***	-0.28	-0.34
	[Mg]	**-0.49***	**-0.40***	**-0.39****	**-0.81***	**-0.57***	-0.31
	[S]	**-0.38****	**-0.37***	-0.17	**-0.81***	-1.62	-0.36
Micronutrients	[B]	**-0.20****	**-0.57***	0.01	**-0.62****	0.91	-0.03
	[Cu]	**-0.14****	**-0.46***	**-0.21***	**-0.45***	1.77	**-0.50****
	[Fe]	-0.09	-0.10	-0.06	-0.32	-0.3	**-0.53***
	[Mn]	0.05	**-0.14***	0.07	0.26	0.12	0.09
	[Zn]	-0.08	**-0.31***	-0.12	0.12	0.14	**-0.38***

Table

Table 2. Standardized Major Axis (SMA) regressions for evaluation of the relationships of leaf nitrogen (N) with other leaf nutrients, for the total pool or for the different forest habitats. The value of the regression slope (the scaling exponent from major axis regression), significance level (°*P*<0.07, *P < 0.05, **P < 0.01, ***P < 0.001) and number of cases used (n) are also shown. Bold and italic values indicate significant relationships that were not consistent with linear mixed models, which suggests that these significant results were highly dependent on some specific species.

log10 (X)	log10 (Y)	Total pool n(172)	SF n(41)	EOF n(55)	PIF n(21)	DOF n(29)	RIF n(26)
[C]	[N]	-4.56***	-0.22**	-6.19	-4.75	3.3	-3.53**
[N]	[P]	0.83***	1.07*	0.77**	0.46*	-0.89	1.00
[N]	[Ca]	2.18***	1.50***	-1.82	3.88°	-2.46	1.59
[N]	[K]	1.41***	1.02***	1.35**	2.01*	1.60	1.31
[N]	[Mg]	1.75***	0.33**	*1.68**	2.91*	1.52	1.33
[N]	[S]	1.39***	0.24**	1.05***	2.01***	0.89	1.20**
[N]	[B]	1.93***	2.43***	-1.95	3.50°	1.69	2.05
[N]	[Cu]	2.48*	0.74	1.34***	4.24***	4.01	2.47
[N]	[Fe]	2.71***	50.6	1.94	6.71	*1.47**	1.56
[N]	[Mn]	2.90	-4.81	-3.85	4.65**	*3.82***	2.13
[N]	[Zn]	2.19	2.78	-2.5	-3.62	2.28	1.81