

1 Foliar elemental composition of European forest tree species associated with
2 evolutionary traits and present environmental and competitive conditions.

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30 ABSTRACT

31 **Aim** Plant elemental composition and stoichiometry is crucial for plant structure and
32 function. We studied to what extent plant stoichiometry might be caused by
33 environmental drivers and competition from coexisting species.

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35 **Location** Europe

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37 **Methods** We analyzed foliar N, P, K, Ca and Mg concentrations and their ratios among
38 50 species of European forest trees sampled in 5284 plots across Europe and their
39 relationships with phylogeny, forest type, current climate and N deposition.

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41 **Results** Phylogeny is strongly related to overall foliar elemental composition in
42 European tree species. Species identity explained the 56.7 percent of the overall foliar
43 elemental composition and stoichiometry. Forest type and current climatic conditions
44 also partially explained the differences in foliar elemental composition among species.
45 In the same genus co-occurring species had overall higher differences in foliar
46 elemental composition and stoichiometry than the non co-occurring species.

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48 **Main conclusions** The different foliar elemental compositions among species are
49 related to phylogenetic distances, but they are also related to current climatic
50 conditions, forest types, global change drivers such as atmospheric N deposition, and
51 to differences among co-occurring species as a probable consequence of niche
52 specialization to reduce direct competition for the same resources. Different species
53 have singular “fixed” foliar elemental compositions but retain some degree of plasticity
54 to the current climatic and competitive conditions. A wider set of elements beyond N
55 and P better represents the biogeochemical niche and is highly sensitive to plant
56 function. Foliar elemental composition can thus be useful for representing important
57 aspects of plant species niches.

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59 **Keywords**

60 Calcium, Ca:Mg, competition, biogeochemical niche, ecological stoichiometry, forests,
61 magnesium, nitrogen, N:K, N:P, phosphorus, P:K, phylogeny, potassium

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87 **INTRODUCTION**

88 Plant elemental composition and stoichiometry is crucial in plant structure and function.

89 (Sterner & Elser, 2002, Sardans *et al.*, 2012a). Various plant structures and metabolic
90 processes have distinct and divergent requirements for each of the essential nutrients.

91 Therefore, one could expect individual species, each an original product of a singular
92 evolutionary history under specific environmental conditions leading to a determined
93 life strategy, to have its own optimal elemental balance, i.e. an optimal stoichiometry.

94 The recently proposed biogeochemical niche hypothesis (Peñuelas *et al.*, 2008; 2010)

95 claims that each species has an optimal elemental composition and stoichiometry as a
96 result of its optimal function in its specific ecological biogeochemical niche. This

97 optimal elemental composition results from the differences in metabolic and
98 physiological functions and morphologies, developed over a long period of time

99 resulting in each species tending to reach an optimum chemical composition linked to a
100 singular optimum function (homeostasis). In addition, plant species should have, to

101 some degree, a flexible adaptation capacity to alter their elemental stoichiometries in
102 response to changes in the composition of neighboring species and/or in

103 environmental conditions (such as climate gradients) (Sardans & Peñuelas, 2013,
104 2014a). This flexibility should result from both a long-term adaptative acquired trait

105 (genotype), but also to genotype expression mechanisms (phenotype). Species are
106 nonetheless expected to exhibit a certain degree of stoichiometric flexibility to be able

107 to respond to environmental changes and competition, probably with a tradeoff
108 between adaptive capacity (flexibility) and stability (homeostasis) (Yu *et al.*, 2010).

109 The anthropogenic deposition of atmospheric N in European forests has
110 induced N saturation in many forests and has even affected the concentrations of

111 several other elements in plants (Sardans *et al.*, 2012b). We, thus, also hypothesized
112 that the deposition of atmospheric N has become another factor that can affect the

113 foliar elemental composition in European forests due to the flexibility of different

114 species to cope with new environmental conditions. Moreover, different elemental
115 compositions and stoichiometric uses of elements among co-occurring species should
116 help to reduce competition or to respond to rapidly changing environmental conditions.
117 We, thus, hypothesized that species that coexist in a climatic area and consequently
118 are more likely to compete with each other will tend to have different foliar elemental
119 compositions even when they are closely phylogenetically related.

120 We consequently hypothesized that different forest species have different foliar
121 elemental compositions (here represented by foliar N, P, K, Ca and Mg concentrations
122 and N:P, N:K, P:K, N:Ca, P:Ca, K:Ca, N:Mg, P:Mg, K:Mg and Ca:Mg ratios). Foliar
123 elemental composition and stoichiometry should be related to different variables: first,
124 they should have a strong genetical signal due to the long-term adaptation of each
125 species to specific abiotic and biotic environments. Each species should have
126 optimized metabolic and physiological functions and morphological structures that
127 determine the specific use of the different nutrients. Thus, distant taxonomic groups
128 should have different elemental composition and stoichiometry. Second, an optimum
129 stoichiometry for each climatic condition should be determined in part by the plant
130 uptake and use efficiency of the different nutrients, effect linked to the different trade-
131 offs among different plant functions that maximize plant fitness in each particular
132 climate situation. Thus, different species sets growing in different forest types under
133 different climatic conditions would tend to have different elemental composition and
134 stoichiometry. Third, the long-term loadings of N in some parts of the world, such as in
135 several European zones, could become an increasingly important factor in determining
136 foliar elemental composition and stoichiometry of forest vegetation. Finally, we also
137 hypothesized the existence of some level of differences in foliar composition
138 stoichiometry among co-occurring species to avoid competition pressure in the use of
139 resources. All this should be also related to the need of some degree of homeostasis
140 capacity but also of flexibility in species-specific elemental composition and
141 stoichiometry. The trade-off between the adaptation to be competitive in more stable

142 environments versus to be successful in more instable ones should be underlying the
143 differences in the continuum homeostasis-flexibility strategy in foliar elemental
144 composition and stoichiometry among different species. This would be consistent with
145 recent observations of species with higher stoichiometry flexibility having higher
146 concentrations of N and P and lower N:P ratios (Yu *et al.*, 2011).

147 We tested these hypotheses by studying the relationships of the foliar elemental
148 compositions and stoichiometries of species with (i) their phylogenetic signal, (ii) forest
149 type, (iii) current climate conditions and (iv) atmospheric N deposition, and finally (v) we
150 tested whether potentially competing tree species of the same forest type can have
151 divergent foliar biogeochemical niches, using a large data set of forest species (n=50)
152 sampled in >5000 plots across Europe.

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171 **METHODS**

172 **Foliar data**

173 We used data from the Catalan Forest Inventory (Gracia *et al.*, 2004), the Third
174 Spanish National Forest Inventory (Villanueva, 2005) and the level II network operated
175 under ICP Forests (International Co-operative Programme on Assessment and
176 Monitoring of Air Pollution Effects on Forests, <http://icp-forests.net/page/data-requests>)
177 established under the Convention on Long-range Transboundary Air Pollution
178 (CLRTAP) of the United Nations Economic Commission for Europe. The sample
179 analyzed for each plot was a single analysis coming from a mixture of samples
180 obtained by mixing leaf samples of at least five leaves in the ICP Forests and three
181 leaves in the Spanish National Forest Inventory of the dominant species of the plot.
182 They were collected at different directions of the crown. All these data had been
183 obtained using comparable analytical methods to analyze leaves. N was analyzed by
184 Kjeldahl, dry combustion and chromatographic methods, and P, K, Ca and Mg were
185 analyzed by atomic spectrometric emission. Foliar N:P:K:Ca:Mg concentration ratios
186 were calculated on the basis of mass. The nutrient concentrations of the same species
187 in the same geographical areas of different databases were analyzed, and no
188 significant differences were observed. Data from a total of 5284 sites were used in the
189 analyses. All foliar samples had been collected in 1990-2006, and the leaves had been
190 fully expanded in all cases. We only used data from plots with known geographical
191 coordinates. All georeferenced data were processed using MiraMon 6.0 (Pons, 2009).
192 The distribution of the plots analyzed is shown in Figure 1. Unfortunately soil data were
193 not available and therefore soil variables have not been included in this analysis.

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195 **Climatic data**

196 We analyzed mean annual temperature (MAT), mean annual precipitation (MAP),
197 annual thermal amplitude, precipitation of the wettest month, precipitation of the driest

198 month, temperature of the warmest month and temperature of the coldest month
199 derived from the WorldClim database (Hijmans *et al.*, 2005), which has a resolution of
200 approximately 1 km² (at the equator). This climatic model is based on interpolated
201 values of climatic data provided by weather stations throughout the territory and
202 adjusted to the observed topography. MAT and MAP were calculated in this climatic
203 model from a long time series of weather (1950-2000).

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205 **N-deposition data**

206 The data for the deposition of atmospheric N were obtained from the European
207 Monitoring and Evaluation Programme (EMEP) of the Convention on Long-range
208 Transboundary Air Pollution CLRTAP. The EMEP MSC-W chemical transport model of
209 this program (Simpson *et al.*, 2012) has been developed to estimate regional
210 atmospheric dispersion and deposition of acidifying and eutrophying compounds (S
211 and N). A detailed description of the model is provided in Simpson *et al.* (2012). For
212 our study, total atmospheric N deposition over Europe was estimated for 2005 with the
213 EMEP model rv3.8.1 using a grid size of 50 × 50 km (EMEP, 2011).

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215 **Phylogenetic and statistical analyses**

216 *Species foliar composition and stoichiometry and their relationships with phylogenetic*
217 *distances*

218 We constructed a phylogenetic tree and obtained the phylogenetic distances among
219 species with Phylomatic and Phylocom (Webb & Donoghue, 2005; Webb *et al.*, 2008).
220 Briefly, Phylomatic uses a backbone plant megatree based primarily on DNA data from
221 a variety of studies to assemble a phylogenetic tree for the species of interest. Our
222 phylogenetic hypothesis was based on the conservative megatree, where unresolved
223 nodes were included as soft polytomies (Webb and Donoghue, 2005). We used the
224 *ape* (Paradis *et al.*, 2004) and *picante* (Kembel *et al.*, 2010) libraries from R software

225 (R Development Core Team, 2011) to test for phylogenetic signals among the foliar
226 elemental composition of the species studied and therefore to determine the extent to
227 which foliar N, P, K, Ca and Mg concentrations, N:P, N:K, P:K, N:Ca, P:Ca, K:Ca, N:Mg,
228 P:Mg, K:Mg and Ca:Mg ratios and PCA component scores had phylogenetic signals.
229 We used the *phylosignal* function of the *picante* package that calculates a statistic of
230 phylogenetic signal (Blomberg's *K*) and a *P*-value based on the variance of
231 phylogenetically independent contrasts relative to tip shuffling randomization.
232 Blomberg's *K* can range from 0 to 1 and indicates the strength of the phylogenetic
233 signal in the tested variable; a value close to 1 indicates that most of the variability in
234 the data can be explained by the phylogeny.

235

236 *Foliar composition relationships with forest type, climate and N-deposition gradients*

237 ANOVAs were performed using the foliar concentrations of the nutrients and the N:P,
238 N:K, P:K, N:Ca, P:Ca, K:Ca, N:Mg, P:Mg, K:Mg and Ca:Mg ratios concentration ratios
239 as dependent variables. Forest type (Mediterranean broadleaf deciduous,
240 Mediterranean needleleaf evergreen, Mediterranean broadleaf evergreen,
241 temperate/boreal needleleaf evergreen and temperate/boreal broadleaf deciduous)
242 was used as categorical independent variable. When a phylogenetic signal was
243 detected for the respective dependent variable, we included phylogeny as an additional
244 independent variable in the corresponding statistical analyses. We used the
245 *compar.gee* function of the *ape* library, which performs a comparative analysis using
246 generalized estimating equations and which also returns the *F*- and *P*-values.

247 To study the direct relationships of climate gradients and N deposition with foliar
248 elemental composition and stoichiometry we firstly assessed the univariant analysis by
249 multiple correlations among foliar chemical traits and climatic and N-deposition data,

250 corrections for false-discovery rates were included in the analyses. We tested for
251 normality and homogeneity of the variance prior to the statistical analyses by
252 examining the residuals plots and the normal qq-plots of the linear models. The data
253 were log-transformed if the required conditions were not met. Thereafter, we correlated
254 the climatic and N-deposition data with the PCA scores to analyze the relationships of
255 climate and N-deposition data with overall foliar elemental composition and
256 stoichiometry.

257 A principal component analyses (PCA) and a discriminant functional analysis
258 (DFA) were performed to determine whether the overall nutrient concentrations and
259 N:P, N:K, P:K, N:Ca, P:Ca, K:Ca, N:Mg, P:Mg, K:Mg and Ca:Mg concentration ratios
260 were determined by the various independent variables studied (phylogenetic distance,
261 forest type, climatic conditions, atmospheric N deposition and different species of the
262 same forest type). These PCA and DFA were conducted with all forest types combined
263 to analyze the foliar elemental compositions among different forest types and the
264 phylogenetic signal of the PCA scores.

265

266 *Foliar elemental composition and stoichiometry in co-occurring species*

267 A second PCA was conducted within the group of typical Mediterranean species
268 (Mediterranean broadleaf deciduous, Mediterranean needleleaf evergreen and
269 Mediterranean broadleaf evergreen). And a third PCA was conducted within temperate
270 and boreal species (temperate/boreal needleleaf evergreen and temperate/boreal
271 broadleaf deciduous) to study the degree of biogeochemical niche segregation among
272 species of the same forest type that frequently compete. We also used one-way
273 ANOVAs to assess whether the PCA scores of the first and second components
274 differed among forest types.

275 Both ordination analyses, PCA and DFA, are complementary (Stamova *et al.*,
276 2009). DFA is a supervised statistical algorithm that will derive an optimal separation
277 between groups established a priori by maximizing between-group variance while
278 minimizing within-group variances (Raamsdonk *et al.*, 2001), whereas PCA does not
279 maximize between-groups variation against within-group variance. We conducted
280 regressions between the log of the PCA-score distances between all pairwise species
281 with the log of phylogenetic distances between all pairwise species. We also conducted
282 regression analysis between the log of the squared Mahalanobis distances between all
283 pairwise species and the log of the phylogenetic distances between all pairwise
284 species. Regressions of the PCA scores of the first and second components with
285 climatic variables and N-deposition levels were conducted to detect possible
286 relationships of biogeochemical niche with climatic variables and N deposition. When
287 needed, variables were log-transformed to normalize their distribution of residuals. We
288 used the Bonferroni post-hoc test in all ANOVAs to discern which forest types or
289 species differed significantly. All the ANOVA, PCA and DFA analyses were performed
290 using StatView 5.0.1 (SAS Institute Inc., Berkeley Ca, USA) and Statistica 6.0 (StatSoft,
291 Inc. Tule, Oklahoma, USA), and the phylogenetic analyses were conducted with R
292 (Development Core Team, 2011).

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302 **RESULTS**

303 **Phylogenetic signals of elemental concentrations**

304 Mean \pm S.E. of the studied variables for each species are shown in the Table S1.
305 Statistically significant phylogenetic signals were detected for the foliar concentrations
306 of most elements, namely N, K, Ca and Mg (Table 1). Surprisingly, P was the only
307 element that did not have a phylogenetic signal (Table 1). Foliar N:P, N:K, P:K and
308 Ca:Mg ratios also exhibited no phylogenetic signal, whereas P:Ca, K:Ca, P:Mg and
309 K:Mg exhibited phylogenetic signal (Table 1). The scores of the PC1 components of
310 the PCA analysis (conducted on the entire data set) also had phylogenetic signals
311 (Table 1). Among the climatic variables, only MAP had a phylogenetic signal (Table 1).

312 The positions of the various species along the biplot of PC1 (explaining 25.7%
313 of the total variance) and PC2 (explaining 21.7% of the total variance) axes strongly
314 coincided with the distribution of the main plant families in the phylogenetic tree (Figure
315 2a). The species belonging to the five families with the most species were separated
316 along these two PCA components. Only *Cupressaceae* relative to *Fagaceae*, and
317 *Cupressaceae* relative to *Betulaceae*, were not significantly separated by the first two
318 PCA components. These families, however, were separated along the PC3 component
319 (explaining 18.2% of the total variance) ($P < 0.0001$ in both cases) (data not shown).
320 The data thus show that foliar elemental composition has a strong phylogenetic signal
321 and consequently that much of the variability in European species-specific foliar
322 elemental composition is explained by the strength of the phylogenetic link among the
323 species. The log of the differences in the PC1 scores between species and the log of
324 the squared Mahalanobis distances between species were correlated with the log of
325 the phylogenetic distances between species ($R = 0.25$, $P < 0.0001$; and $R = 0.45$,
326 $P < 0.0001$, respectively) (Figure S1).

327

328 **Differences among the forest types**

329 Mediterranean evergreen forests, both broadleaf and needleleaf, are located in areas
330 with generally lower MAPs and higher MATs. Mediterranean deciduous forests are
331 located at intermediate locales, while temperate/boreal forests, both evergreen and
332 deciduous, exhibit the highest MAPs and lowest MATs (Table S2). The Mediterranean
333 broadleaf deciduous and temperate/boreal broadleaf deciduous forests had the highest
334 foliar concentrations of most elements (Figure 3). Only Mg concentrations were higher
335 in Mediterranean needleleaf evergreen than in temperate/boreal broadleaf deciduous
336 forests (Figure 3). In contrast, Mediterranean needleleaf evergreen forests had the
337 lowest N, P and K foliar concentrations (Figure 3), whereas temperate/boreal
338 needleleaf forests had the lowest Ca and Mg foliar concentrations. Mediterranean
339 broadleaf evergreen forests had intermediate foliar concentrations for all five elements
340 studied (Figure 3). Needleleaf forests generally had the lowest foliar K concentrations
341 and the highest N:K and P:K ratios (Figure 3). Interestingly, temperate/boreal
342 needleleaf forests had the lowest foliar N:P ratios, coinciding with the presence in this
343 group of economically important fast-growing species such as *Picea abies*.
344 Surprisingly, however, Mediterranean needleleaf forests had the lowest foliar Ca:Mg
345 ratios (Figure 3). A comparison of the overall foliar composition in a PCA indicated that
346 all forest types were separated in the ordination space formed by the first three
347 components (Figure 4). All the forest types were separated respect the others at least
348 across 2 of the first three components and all them were separated along the first
349 component. The variables with the highest loadings on the first three PCA components
350 were foliar P, Ca and Mg concentrations and N:Ca, P:Ca, K:Ca, N:Mg, P:Mg and K:Mg
351 ratios, with needleleaf forests located toward lower foliar N, P and K concentrations
352 and higher N:K and P:Ca ratios, and with wet/temperate broadleaf forests toward
353 higher foliar N and K concentrations and K:Mg and Ca:Mg ratios. The DFA analysis
354 further confirmed the results of the PCA, showing that the squared Mahalanobis
355 distances between all forest types were significantly different (Table S3) and that all the

356 foliar elemental concentrations and ratios used in the DFA were statistically significant
357 in the model (Table S4).

358

359 **Effects of climate and atmospheric N deposition**

360 MAP was significantly and positively correlated with foliar P and K concentrations,
361 whereas MAT was mainly correlated negatively with foliar N, P and K concentrations
362 and positively with foliar Mg concentrations (Table 2). Atmospheric N deposition was
363 correlated with higher foliar N and Ca concentrations, higher N:P ratios and lower foliar
364 P and Mg concentrations (Table 2). The effects of atmospheric N deposition on overall
365 elemental composition, as determined by the correlations with PCA scores, were lower
366 (N deposition was only correlated with the scores of the third principal component)
367 (Table 2).

368

369 **Species differences in foliar elemental composition and stoichiometry**

370 When all Mediterranean and temperate/boreal species were analyzed in a DFA, nearly
371 all pairwise comparisons had significantly different Mahalanobis distances (data not
372 shown). Only some species of the same genus, such as *Pinus radiata* relative to *P.*
373 *sylvestris*, *P. pinaster* and *P. nigra* or *Q. petraea* relative to *Q. canariensis*, did not have
374 significant squared Mahalanobis distances. The canonical *R* values of the first and
375 second roots were 0.87 (Figure 5) and 0.70, and species identity explained 56.7% of
376 the overall foliar composition and stoichiometry variance.

377 When only data from non-Mediterranean species were analysed by PCA, all
378 pairwise species comparisons of PC axes scores were statistically different at least in
379 one of the first four axes (Figure 6). All Mahalanobis distances between pairwise
380 species were significant in a DFA analysis conducted only with the non-Mediterranean
381 species (Table S5) being all the variables significant ($P < 0.0001$) in the DFA model
382 separating non-Mediterranean tree species (Table S6). The canonical *R* values of the
383 first and second roots were 0.88 (Figure S2a) and 0.66, and species identity explained

384 the 64.3% of the overall foliar composition and stoichiometry variance. Similar results
385 were observed in the corresponding PCA in Mediterranean species where all pairwise
386 species comparisons of PC axes scores were statistically different at least in one of the
387 first four axes (Figure 7). All Mahalanobis distances between pairwise species were
388 significant in a DFA analysis conducted only with the Mediterranean species that
389 frequently compete (except between *Arbutus unedo* and *Eucalyptus* sp.) (Table S7).
390 Foliar N, P, K, Ca and Mg concentrations and all their ratios were significant
391 ($P < 0.0001$) in the DFA model separating Mediterranean tree species (Table S8). The
392 canonical R values of the first and second roots were 0.87 (Figure S2b) and 0.70, and
393 species identity explained 51.8% of the overall foliar composition and stoichiometry
394 variance.

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412 **DISCUSSION**

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414 **Foliar elemental composition and phylogeny**

415 As we hypothesized, the overall foliar elemental composition showed a strong
416 phylogenetic signal. The differences in foliar elemental composition among different
417 species increased with phylogenetic distance. Genetic differences among species
418 result from long-term adaptation under species-specific environmental conditions that
419 selected for particular metabolic functions. However, the long-term evolutionary
420 differences (e.g. needles versus leaves, or within angiosperms long-lived evergreen
421 versus summer green leaves) could, at some extent, determine different
422 morphological-functional adaptative solutions in front of the same environment, thus
423 being a constraint in further evolutionary processes. In any case, the studied elements
424 such as N, P and K have well defined functions in all higher plants, for example both
425 angiosperms and gymnosperms need more P to accelerate its protein synthesis, or
426 more K to better control stomata openness. Thus, it is very likely that in most higher
427 plant species similar optimal functions are related to similar concentrations of the most
428 functional elements, such as those investigated in this study.

429 Previous stoichiometric studies comparing foliar elemental composition of plants
430 of different taxonomic groups and forest types focused mainly on the concentrations
431 and ratios of C, N and P (Powers & Tiffin, 2010) or of N and P (Han *et al.*, 2005; Stock
432 & Verboom, 2012). These studies have reported phylogenetic differences in foliar N
433 and P concentrations (Powers & Tiffin, 2010; Stock & Verboom, 2012) and among
434 different functional types (Han *et al.*, 2005). Other studies, however, have only found
435 phylogenetic signals in foliar N when legumes were included (Powers & Tiffin, 2010). In
436 fact, when analyzed separately, the foliar concentration of all elements had a
437 significant phylogenetic signal, and only P did not, perhaps because plants tend to
438 abundantly use P opportunistically. When plant growth is limited by P, plants invest in
439 the production of phosphatases, energetically expensive compounds that are only

440 secreted by plants in P-limited conditions (Treseder & Vitousek, 2001). Energy flows
441 extensively to the rhizosphere in the absence of P fertilization and is used by the
442 microbial community (Johnson *et al.*, 1997) but stops following P fertilization. Assuming
443 that differences among rhizospheric microbial communities are a consequence of
444 differences in the patterns of exudation from plants (Marschner & Timonen, 2005), the
445 impact of exudation on the structure of microbial communities must be more
446 pronounced in the absence of P fertilization, which is likely a general plant trait
447 throughout the phylogenetic spectrum. Moreover, the principal paradigmatic ratio in
448 ecological stoichiometry, the N:P ratio, showed no phylogenetic signal. P seems to be
449 determined by current climatic conditions and also by a recent phenomenon in Europe,
450 the N deposition, which can disturb the natural foliar P concentrations and the capacity
451 to detect its phylogenetical signal. N:P did not show phylogenetical input probably due
452 to the lack of P phylogenetical signal and/or due to the variability generated by climate
453 and N deposition. Moreover, several studies have shown that the foliar concentrations
454 of these two elements tend to scale linearly with a slope lower than one and that this
455 relationship is similar in different vegetation types and taxonomic groups (Wright *et al.*,
456 2004). Anyway, the studied trees of the *Pinaceae* family, which are mostly fast-growing
457 species (Gracia *et al.*, 2004; Villanueva, 2005), have on average the lowest foliar N:P
458 ratios, as expected in the theories of ecological stoichiometry such as the growth rate
459 hypothesis. Our results, though, show that the use of a more comprehensive set of
460 nutrients enhances the sensitivity of detecting differences at the phylogenetic level, but
461 also among forest types and among species growing within the same forest type. The
462 PCAs using both the foliar concentrations and ratios of several elements clearly have a
463 higher sensitivity for detecting phylogenetic signals than do the PCAs analyzing
464 individual elements, suggesting that overall elemental composition and stoichiometry
465 are strongly associated with the long-term evolution of species in their specific
466 environments.

467 Variability in the availability of soil nutrients can contribute to the variation
468 observed in foliar stoichiometry (Paoli, 2005; Stock & Verboom, 2012). Stock and
469 Verboom (2012) studied the phylogenetic signal of foliar N and P concentrations and
470 N:P ratios in a data set of 564 plant species in various areas of the Mediterranean
471 Basin and observed that different soils explained a large proportion of the variance in
472 the foliar concentrations of these elements. Unfortunately, the Catalan and Spanish
473 forest inventories do not include soil data, and the soil data in the ICP forest database
474 did not generally coincide with the foliar chemical data in the same plots, so we had
475 insufficient information to include soil variables in our statistical analyses. Anyway, soil
476 differences depend of bedrock but also of climatic conditions, with similar bedrocks
477 providing very different soils and soil nutritional status under different climate
478 conditions (Soil Survey Staff, 1998). Moreover, the results show that bedrock type is
479 not necessarily the most important factor in explaining the differences in foliar
480 elemental composition and stoichiometry among the studied species. For example, the
481 squared Mahalanobis distances of elemental composition and stoichiometry between
482 the Mediterranean pines *P. halepensis* and *P. nigra*, both with preference for growing
483 on calcareous bedrocks, are 72.2, larger than the observed distances between *P.*
484 *halepensis* and *P. pinaster* (16.3) or *P. pinea* (31.6), both growing preferentially on
485 plutonic acid rocks (Table 5). Similarly, within Mediterranean *Quercus*, *Q. suber*
486 growing preferentially over plutonic acid rocks has more similar squared Mahalanobis
487 distances of elemental composition and stoichiometry with respect to *Q. faginea* (17.4)
488 growing preferentially over calcareous rocks than with respect to *Q. ilex* (23.3) growing
489 over a broad spectrum of substrates (Table 5).

490

491 **Foliar elemental composition and stoichiometry in different forest types, and**
492 **climate and N-deposition gradients.**

493 We also observed significant differences in foliar elemental composition among forest
494 types growing in different climatic conditions. For example, Mediterranean evergreen

495 broadleaf and temperate/boreal deciduous forests, both groups dominated by trees of
496 the *Fagaceae* family, had strikingly different foliar concentrations of the five elements
497 analyzed and different foliar ratios such as N:P, N:K and Ca:Mg. Our results are also
498 consistent with previous studies that have observed that species in different climatic
499 areas or under different levels of atmospheric N deposition have different foliar
500 elemental compositions (Oleksyn *et al.*, 2003; Reich & Oleksyn, 2004; Sardans *et al.*,
501 2011;2013 Sardans & Peñuelas 2014b). Thus, our study demonstrates that
502 taxonomical differences account to explain a significant part of the differences in foliar
503 elemental composition among European forest species but also that genotypic and
504 phenotypic response to environmental conditions contribute to some degree to
505 changes in foliar elemental composition.

506

507 **Foliar elemental composition and stoichiometry among co-occurring species**

508 We observed that even species of the same genus and co-occurring in the same forest
509 type have different foliar elemental compositions, indicating competition and niche
510 separation. Whether the different chemical compositions among species that frequently
511 compete during at least some phases of their lives are the cause or the consequence
512 of avoidance of competition is difficult to determine. Changes in plant function and/or
513 structure to reduce competition, such as growing roots in different soil compartments or
514 different strategies of capturing light, are probably inseparable from differences in the
515 ability to capture and/or use resources (Mamolos *et al.*, 1995). Changes in plant
516 function and elemental composition are thus very probably mutually dependent. In fact,
517 some degree of differentiated function is observed in plants in the same community,
518 e.g. they tend to dominate in different successional stages and/or different
519 microclimatic and edaphic conditions or exploit different light spectra (Ellsworth &
520 Reich, 1996; Garnier *et al.*, 2004).

521

522 **Specific-species foliar elemental composition: the foliar biogeochemical niche**

523 These results provide solid evidence that the differences in the function and structure
524 of species are linked to differential use of bioelements, supporting the idea of the foliar
525 biogeochemical niche as the place occupied in the multivariate space generated by the
526 concentrations of macro- and micronutrients and their stoichiometric relationships
527 (Peñuelas *et al.*, 2008, 2010). Our results further indicate that the species-specific foliar
528 biogeochemical niche cannot be exclusively defined by only one factor but should have
529 two dimensions. First, the evolutionary-genotypic dimension: the differences in
530 metabolic and physiological functions and morphologies developed over a long period
531 of time should translate into different proportional uses of nutrients and consequently
532 into different elemental stoichiometries, which should provide some degree of
533 genotypic homeostasis. Second, the flexibility related to both evolutionary processes
534 and phenotypic plasticity to respond to current environmental changes.

535 First, the foliar biogeochemical niche is the result of species specialization and
536 evolutionary processes that lead to adaptations to the global (environmental)
537 conditions (Pärtel *et al.*, 2007), including both abiotic environmental conditions, such as
538 climate, and biotic relationships, such as interspecific competition or interaction with
539 root symbionts. All these adaptative trajectories likely produce a certain range of
540 elemental composition that is typical of a species. In fact, all the variables tested in
541 relation with species-specific foliar elemental composition and stoichiometry are not
542 mutually independent. A phylogenetic signal in elemental composition and
543 stoichiometry should be the result, at least in part, of long-term evolution under long-
544 term climatic conditions, but also under a determined neighbourhood environment.

545 Second, plant species should be able, to some degree, to alter their elemental
546 stoichiometries as a flexible response to changes in the composition of neighboring
547 species and/or in environmental conditions. This flexibility, which can vary among
548 species, can be a consequence of evolutionary processes, and thus have a genotypic
549 component (genotypic flexibility), which should be larger in species that have evolved
550 in environments that are more variable. This second dimension of the foliar

551 biogeochemical niche would be underlying the ability of species to vary their elemental
552 compositions, and merits further study. Plant species all face a trade-off between
553 maximizing stoichiometric homeostasis (and thus being best adapted to a single set of
554 conditions) and allowing some degree of flexibility (and thus being able to do relatively
555 well in a wider set of conditions). Species adapted to nutritionally poor environments
556 and with lower capacities for nutrient uptake and higher nutrient-use efficiencies
557 probably evolved with a more homeostatic stoichiometry and had a lower capacity to
558 change their functioning in response to environmental changes (Aerts, 1999). These
559 species probably had a reduced capacity to alter their elemental composition and
560 would have been less able to exploit nutrient pulses. In contrast, species adapted to
561 nutritionally richer or ruderal environments, where nutrients are intermittently
562 abundantly available, have higher capacities for taking up resources and should
563 therefore have higher stoichiometric flexibility, linked to their higher capacity to respond
564 to the changes in resource availability (Aerts, 1999). The coefficients of variation of the
565 foliar chemical variables studied within the different forest types did not indicate
566 different levels of variation (data not shown), suggesting that the various European
567 forest types have a similar degree of stoichiometric flexibility.

568 The study has been focused on foliar biomasses, and this does not allow
569 providing evidences of changes in total plant stoichiometry. But leaves are the plant
570 organs where most plant compounds (from those allocated to growth or reproduction to
571 those allocated to energetic metabolism, defence or storage) are synthesized.
572 Therefore leaves constitute a crucial organ when plant responds to changes in its
573 functions. In this context, we can not define the plant global elemental composition and
574 stoichiometry but by knowing the value and changes in foliar biomass stoichiometry we
575 can define the “species biogeochemical niche” representing the adequate foliar
576 elemental composition and stoichiometry for the optimal foliar function for a determined
577 species in its environmental circumstances.

578

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584

585 Biosketch

586 Jordi Sardans is a senior researcher at the Global Ecology unit CSIC-CREAF. His main
587 focuses of interest are ecological stoichiometry, ecometabolomics, molecular ecology
588 and biogeochemistry of terrestrial ecosystems. The other coauthors are researchers in
589 global ecology, biogeochemistry and biogeography.

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764 Figure captions

765

766 Figure 1. Distribution of the European forests analyzed. The colours represent the
767 dominant forest types in each area.

768 Figure 2. Plots of the PCA space cases (a) and variables (b), defined by the first two
769 components of the PCA conducted with the foliar N, P, K, Ca and Mg concentrations
770 and N:P, N:K, P:K, N:Ca, P:Ca, K:Ca, N:Mg, P:Mg, K:Mg and Ca:Mg ratios as variables
771 and the 5284 forest sites as cases and by the relationships between PC1 scores and
772 phylogenetic position of the main taxonomic groups. The numbers within the PCA plot
773 for cases correspond to the species. They are correlative in the phylogenetic tree.

774 Arrows in this plot indicate the mean of the scores of the main families along the PC1
775 and PC2 components. Different letters on the arrows indicate significant differences
776 ($P < 0.05$). The colors of the arrows indicate the different families. The figure graphically
777 shows the strong relationships between the phylogenetic distances and the differences
778 in foliar composition among species and families. These relationships are shown in

779 Figure 3. Means \pm SE of the foliar N, P, K, Ca and Mg concentrations and N:P, N:K,
780 P:K and Ca:Mg ratios of the various forest types. Different letters indicate significant
781 differences ($P < 0.05$).

782 Figure 4. Three-dimensional representation of the space defined by the first three PC
783 components of the PCA conducted with foliar N, P, K, Ca and Mg concentrations and
784 N:P, N:K, P:K, N:Ca, P:Ca, K:Ca, N:Mg, P:Mg, K:Mg and Ca:Mg ratios as variables (a),
785 and the 5284 European forests as cases (b). The arrows for plot (b) highlight the mean
786 values of the scores of the various forest types, and different letters on the arrows
787 indicate significant differences ($P < 0.05$).

788 Figure 5. Regression between the observed and species-expected (species mean)
789 canonical scores of the first root of the DFA conducted with all studied species.

790 Figure 6. Representation of the PCA space (cases and variables) defined by the two
791 first PC axes with the foliar N, P, K, Ca and Mg concentrations and foliar and N:P, N:K,

792 P:K, N:Ca, P:Ca, K:Ca, N:Mg, P:Mg, K:Mg and Ca:Mg ratios as variables (a) and the
793 non-Mediterranean forests as cases (b). All species separated by at least one of the
794 first three axes (PC3 explained 14.9% of the total variance). The confidence intervals
795 indicate Standard Errors for each species (n = from 5 to 1100).

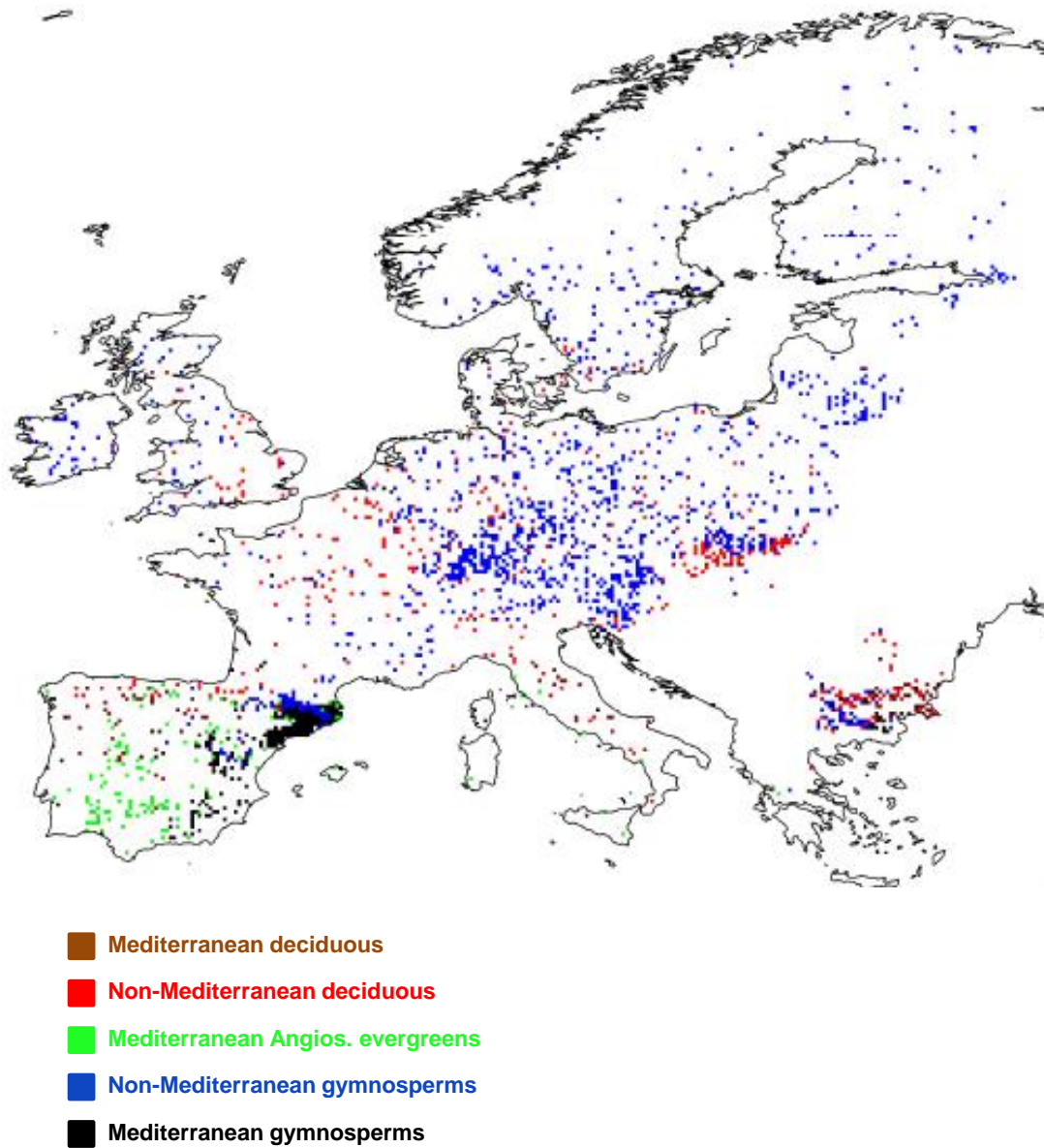
796 Figure 7. Representation of the PCA space (cases and variables) defined by the two
797 first PC axes with the foliar N, P, K, Ca and Mg concentrations and foliar and N:P, N:K,
798 P:K, N:Ca, P:Ca, K:Ca, N:Mg, P:Mg, K:Mg and Ca:Mg ratios as variables (a) and the
799 Mediterranean forests as cases (b). All species separated by at least one of the first
800 three axes (PC3 and PC4 explained 17.2% and 14.8% respectively of the total
801 variance). The confidence intervals indicate Standard Errors for each species (n = from
802 5 to 1100).

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Figure 1

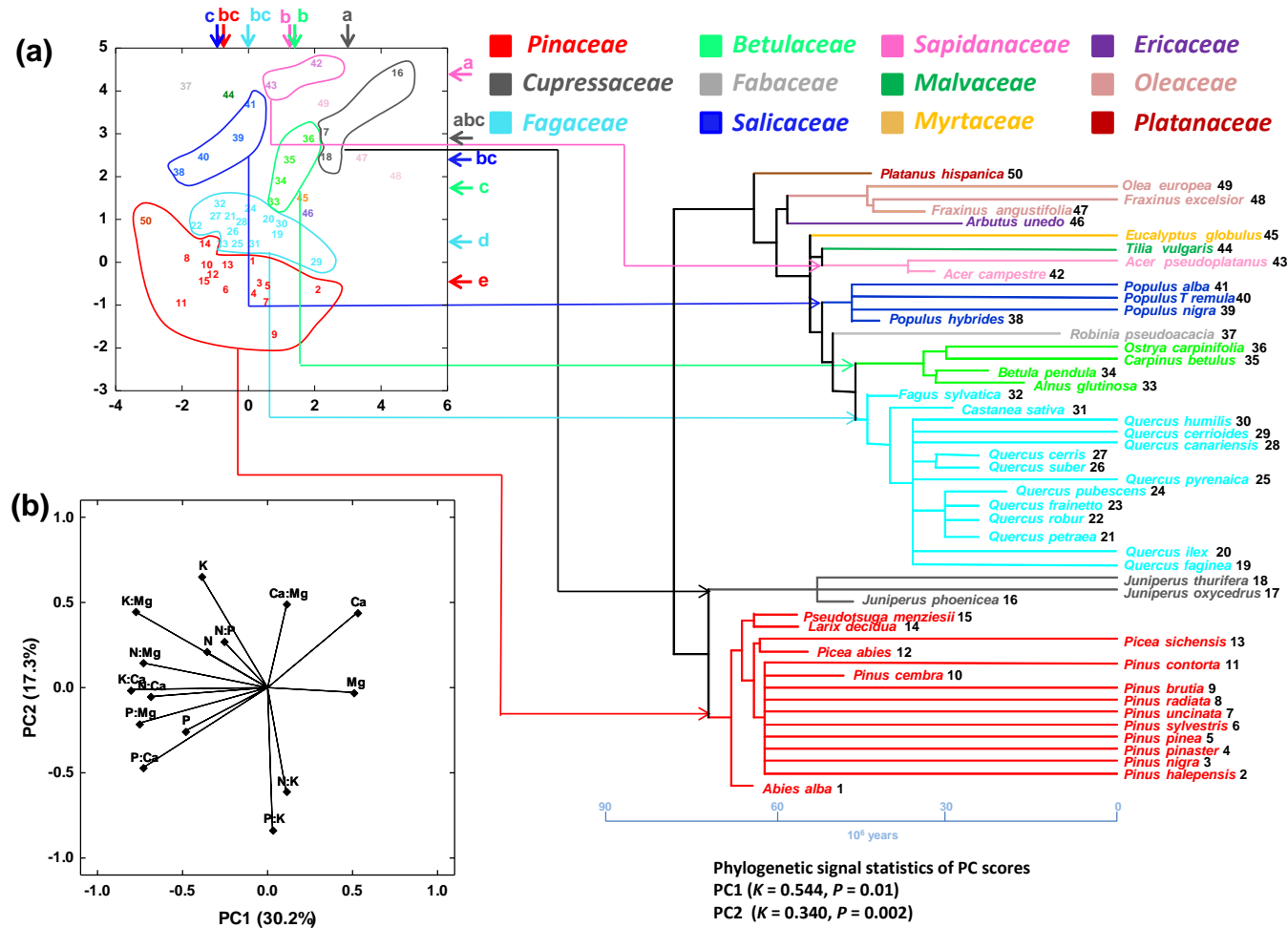


Figure 2

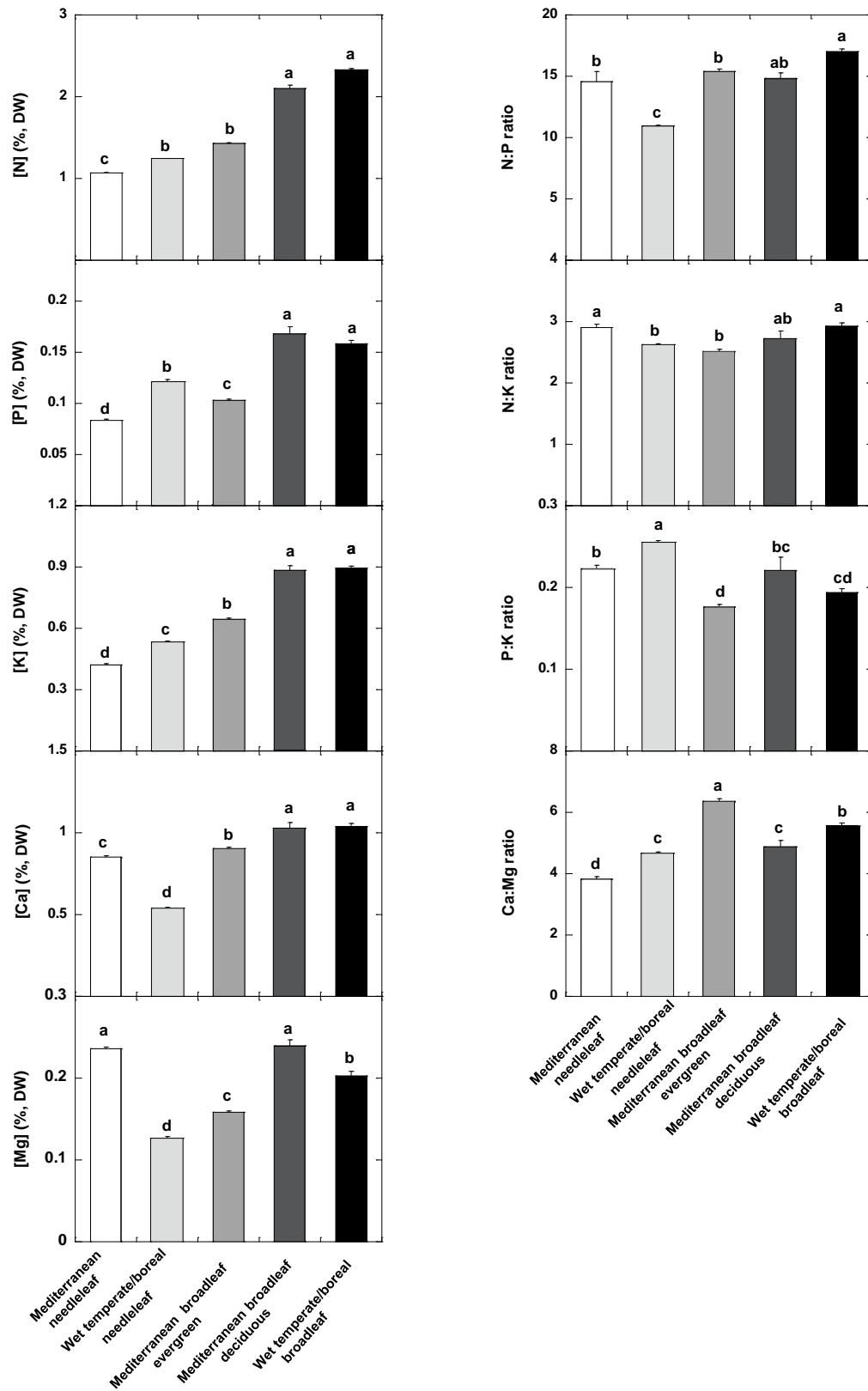


Figure 3

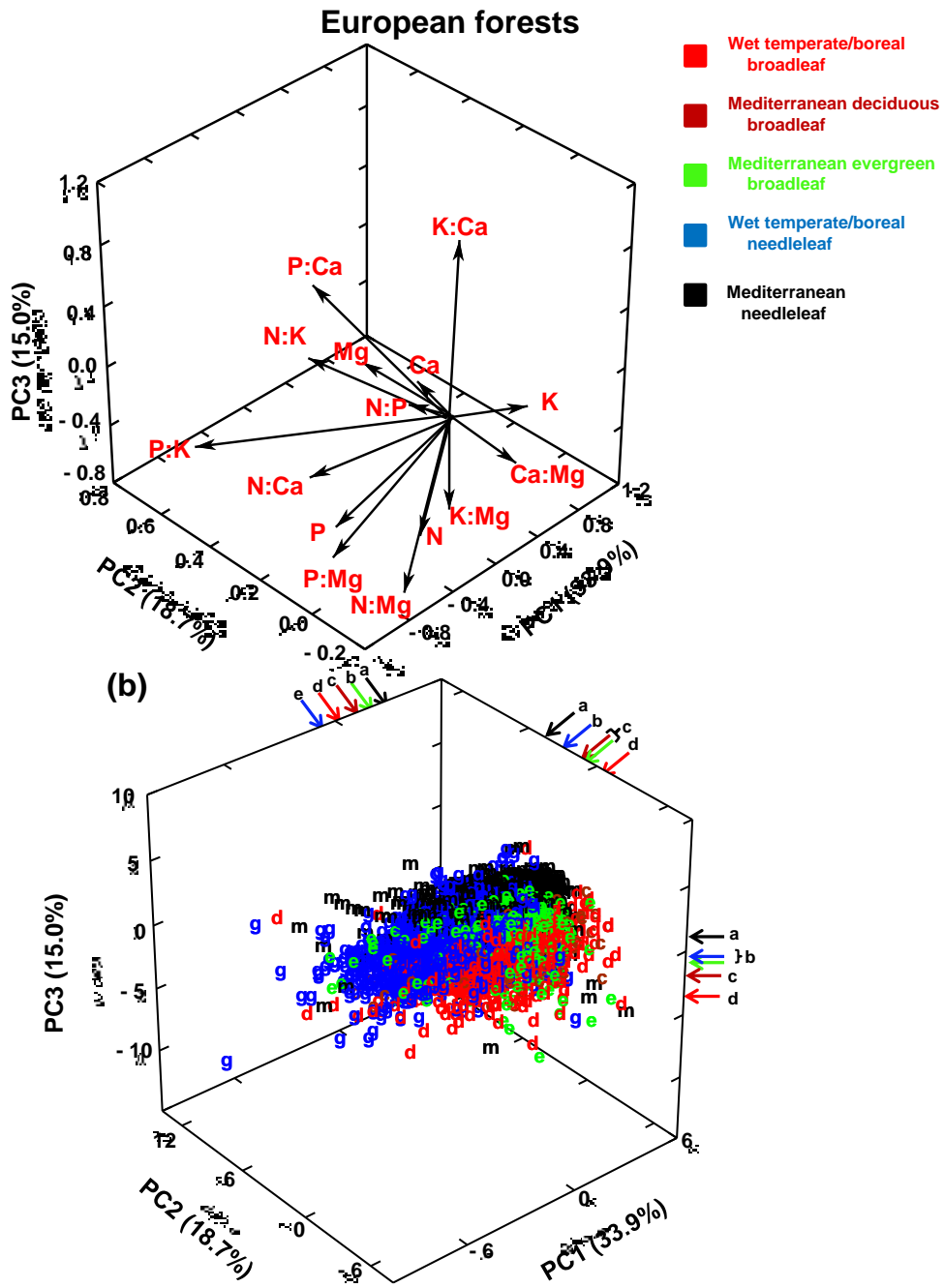


Figure 4

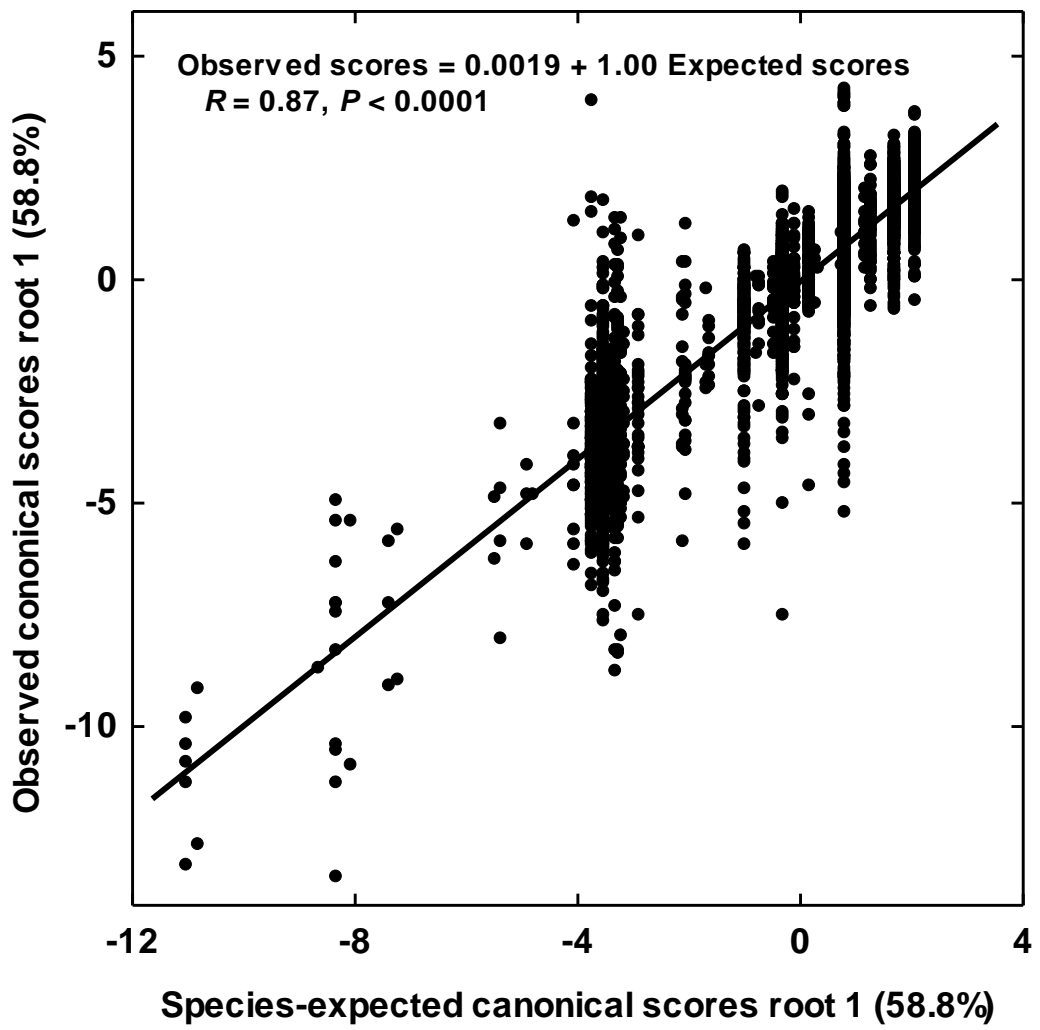


Figure 5

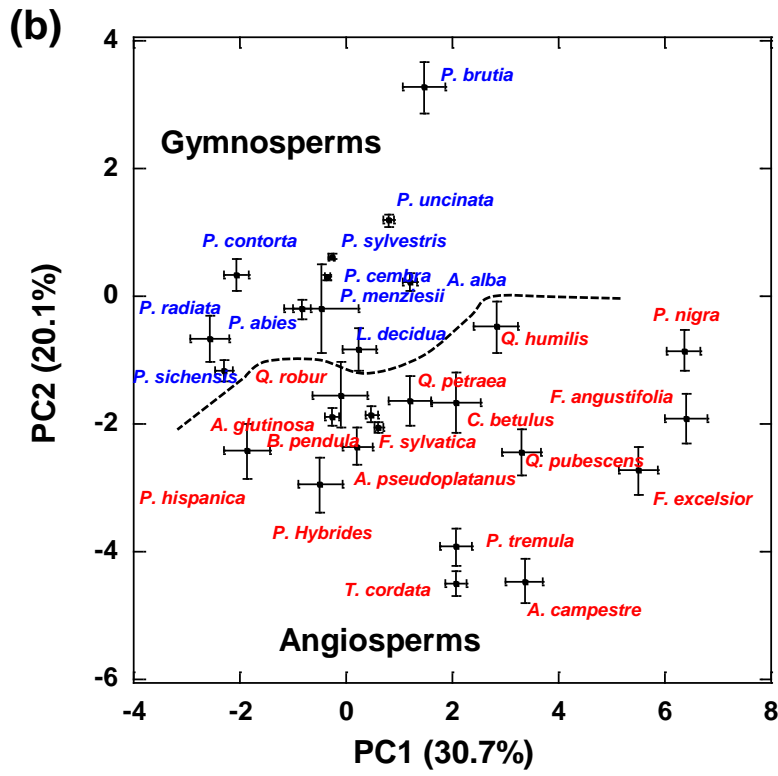
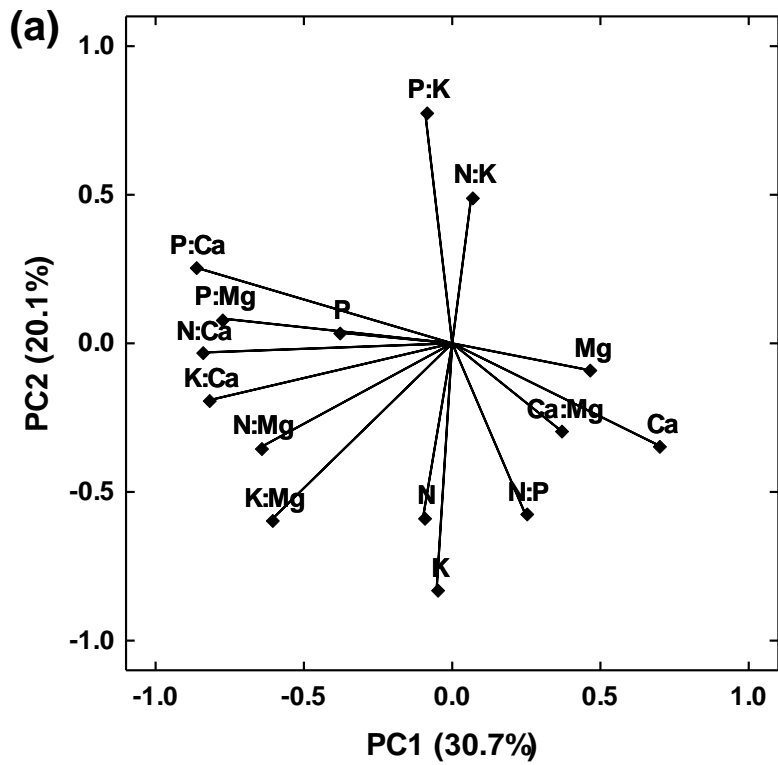


Figure 6

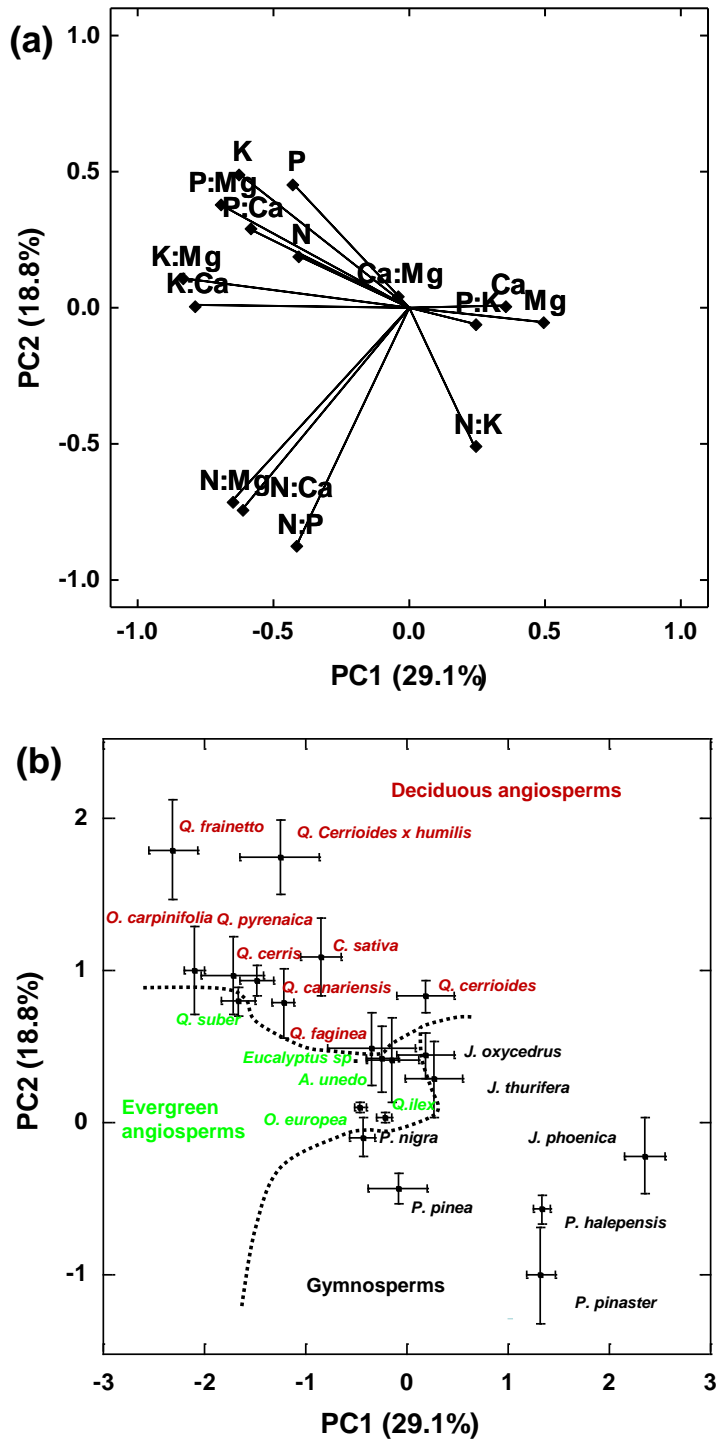


Figure 7

Table 1. Phylogenetic signals (Webb and Donoghue 2005; Webb, Ackerly, and Kembel 2008) of different variables and of PC-axes scores of the PCA conducted with overall foliar elemental compositions (N, P, K, Ca and Mg concentrations and N:P, N:K, P:K and Ca:Mg ratios) of European forests. Benjamini-Hochberg corrections for false-discovery rates were included in the analyses to correct the alpha inflation. The variables with significant ($P < 0.024$) phylogenetic signals are highlighted in bold type. (K=statistic, P=level of significance).

Variable	Phylogenetic signal statistics	
	<i>K</i>	<i>P</i>
Longitude	0.224	0.38
Latitude	0.311	0.094
Foliar [N]	0.443	<0.0001
Foliar [P]	0.361	0.32
Foliar [K]	0.464	0.01
Foliar [Ca]	0.550	<0.0001
Foliar [Mg]	0.502	0.02
Foliar N:P ratio	0.354	0.38
Foliar N:K ratio	0.219	0.42
Foliar P:K ratio	0.258	0.54
Foliar N:Ca ratio	0.347	0.03
Foliar P:Ca ratio	0.487	0.009
Foliar K:Ca ratio	0.465	0.004
Foliar N:Mg ratio	0.347	0.03
Foliar P:Mg ratio	0.487	0.01
Foliar K:Mg ratio	0.465	0.002
Foliar Ca:Mg ratio	0.525	0.39
PC1 (all ratios)	0.426	0.002
PC2 (all ratios)	0.345	0.03
PC3 (all ratios)	0.325	0.04
PC4 (all ratios)	0.527	0.29
PC5 (all ratios)	0.530	0.53
PC6 (all ratios)	0.500	0.12
MAP	0.443	<0.0001
MAT	0.237	0.30
Annual Thermal amplitude	0.245	0.35
Total N deposition	0.369	0.066

Table 2. Correlation coefficients of foliar chemical traits with climatic variables and N deposition. Bonferroni corrections for false-discovery rates were included in the analyses to correct the alpha inflation. Significant differences ($P < 0.001$) are highlighted in bold type.

	MAP	MAT	Annual thermal amplitude	Total N deposition
Foliar [N]	$R=0.019$ $P=0.16$	$R=-0.1$ $P<0.0001$	$R=0.13$ $P<0.0001$	$R=0.064$ $P<0.0001$
Foliar [P]	$R=0.047$ $P<0.001$	$R=-0.26$ $P<0.0001$	$R=0.21$ $P<0.0001$	$R=-0.13$ $P<0.0001$
Foliar N:P ratio	$R=-0.023$ $P=0.089$	$R=0.10$ $P<0.0001$	$R=-0.046$ $P=0.001$	$R=0.10$ $P<0.0001$
Foliar N:K ratio	$R=-0.031$ $P=0.022$	$R=0.019$ $P=0.170$	$R=-0.042$ $P=0.002$	$R=0.092$ $P<0.0001$
Foliar P:K ratio	$R=0.0062$ $P=0.650$	$R=-0.11$ $P<0.0001$	$R=0.034$ $P=0.015$	$R=-0.040$ $P=0.004$
Foliar [Ca]	$R=0.059$ $P<0.0001$	$R=0.17$ $P<0.0001$	$R=-0.14$ $P<0.0001$	$R=0.080$ $P<0.0001$
Foliar [Mg]	$R=-0.11$ $P<0.0001$	$R=0.27$ $P<0.0001$	$R=-0.094$ $P<0.0001$	$R=-0.057$ $P<0.0001$
Foliar [K]	$R=0.076$ $P<0.0001$	$R=-0.069$ $P<0.0001$	$R=0.088$ $P<0.0001$	$R=-0.032$ $P=0.022$
Foliar Ca:Mg ratio	$R=0.17$ $P<0.0001$	$R=-0.073$ $P<0.0001$	$R=-0.050$ $P<0.0001$	$R=0.15$ $P=<0.0001$
PC1 scores	$R=0.070$ $P<0.0001$	$R=-0.0046$ $P=0.736$	$R=0.040$ $P=0.004$	$R=0.0089$ $P=0.516$
PC2 scores	$R=0.031$ $P=0.026$	$R=-0.12$ $P<0.0001$	$R=0.10$ $P<0.0001$	$R=-0.0085$ $P=0.539$
PC3 scores	$R=0.044$ $P=0.001$	$R=0.20$ $P<0.0001$	$R=-0.20$ $P<0.0001$	$R=0.18$ $P<0.0001$
PC4 scores	$R=-0.17$ $P<0.0001$	$R=0.30$ $P<0.0001$	$R=-0.088$ $P<0.0001$	$R=-0.061$ $P<0.0001$

MAP=Mean anual precipitation

MAT=Mean anual temperature