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

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ABSTRACT

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

Location Europe

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

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

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

58 **Keywords**

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

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INTRODUCTION

Plant elemental composition and stoichiometry is crucial in plant structure and function. (Sterner & Elser, 2002, Sardans *et al.*, 2012a). Various plant structures and metabolic processes have distinct and divergent requirements for each of the essential nutrients. Therefore, one could expect individual species, each an original product of a singular evolutionary history under specific environmental conditions leading to a determined life strategy, to have its own optimal elemental balance, i.e. an optimal stoichiometry. The recently proposed biogeochemical niche hypothesis (Peñuelas *et al.*, 2008; 2010) claims that each species has an optimal elemental composition and stoichiometry as a result of its optimal function in its specific ecological biogeochemical niche. This optimal elemental composition results from the differences in metabolic and physiological functions and morphologies, developed over a long period of time resulting in each species tending to reach an optimum chemical composition linked to a singular optimum function (homeostasis). In addition, plant species should have, to some degree, a flexible adaptation capacity to alter their elemental stoichiometries in response to changes in the composition of neighboring species and/or in environmental conditions (such as climate gradients) (Sardans & Peñuelas, 2013, 2014a). This flexibility should result from both a long-term adaptative acquired trait (genotype), but also to genotype expression mechanisms (phenotype). Species are nonetheless expected to exhibit a certain degree of stoichiometric flexibility to be able to respond to environmental changes and competition, probably with a tradeoff between adaptive capacity (flexibility) and stability (homeostasis) (Yu *et al.*, 2010).

The anthropogenic deposition of atmospheric N in European forests has induced N saturation in many forests and has even affected the concentrations of several other elements in plants (Sardans *et al.*, 2012b). We, thus, also hypothesized that the deposition of atmospheric N has become another factor that can affect the foliar elemental composition in European forests due to the flexibility of different species to cope with new environmental conditions. Moreover, different elemental

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

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

differences in the continuum homeostasis-flexibility strategy in foliar elemental composition and stoichiometry among different species. This would be consistent with recent observations of species with higher stoichiometry flexibility having higher concentrations of N and P and lower N:P ratios (Yu *et al.*, 2011).

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

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METHODS

Foliar data

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

Climatic data

We analyzed mean annual temperature (MAT), mean annual precipitation (MAP), annual thermal amplitude, precipitation of the wettest month, precipitation of the driest month, temperature of the warmest month and temperature of the coldest month

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

N-deposition data

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

Phylogenetic and statistical analyses

Species foliar composition and stoichiometry and their relationships with phylogenetic distances

We constructed a phylogenetic tree and obtained the phylogenetic distances among species with Phylomatic and Phylocom (Webb & Donoghue, 2005; Webb *et al.*, 2008). Briefly, Phylomatic uses a backbone plant megatree based primarily on DNA data from a variety of studies to assemble a phylogenetic tree for the species of interest. Our phylogenetic hypothesis was based on the conservative megatree, where unresolved nodes were included as soft polytomies (Webb and Donoghue, 2005). We used the *ape* (Paradis *et al.*, 2004) and *picante* (Kembel *et al.*, 2010) libraries from R software (R Development Core Team, 2011) to test for phylogenetic signals among the foliar

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

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

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

To study the direct relationships of climate gradients and N deposition with foliar elemental composition and stoichiometry we firstly assessed the univariant analysis by multiple correlations among foliar chemical traits and climatic and N-deposition data, corrections for false-discovery rates were included in the analyses. We tested for

normality and homogeneity of the variance prior to the statistical analyses by examining the residuals plots and the normal qq-plots of the linear models. The data were log-transformed if the required conditions were not met. Thereafter, we correlated the climatic and N-deposition data with the PCA scores to analyze the relationships of climate and N-deposition data with overall foliar elemental composition and stoichiometry.

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

Foliar elemental composition and stoichiometry in co-occurring species

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

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

RESULTS

Phylogenetic signals of elemental concentrations

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

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

Differences among the forest types

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

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

Effects of climate and atmospheric N deposition

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

Species differences in foliar elemental composition and stoichiometry

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

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

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

DISCUSSION

Foliar elemental composition and phylogeny

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

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

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

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

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

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

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

Foliar elemental composition and stoichiometry among co-occurring species

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

Specific-species foliar elemental composition: the foliar biogeochemical niche

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

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

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

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

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

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Biosketch

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REFERENCES

- Aerts, R. (1999) Interspecific competition in natural plant communities: mechanisms, trade-off and plant-soil feedbacks. *Journal of Experimental Botany*, **50**, 29-37.
- Ellsworth, D.S., Reich, P.B. (1996) Photosynthesis and leaf nitrogen in five Amazonian tree species during early secondary succession. *Ecology*, **77**, 581-594.
- EMEP (2011) Transboundary Acidification, Eutrophication and Ground Level Ozone in Europe in 2009, EMEP Status Report 1/2011, Joint MSC-W & CCC & CEIP Report, ISSN 1504-6109.
- Garnier, E., Cortez, J., Billes, G., Navas, M.L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C., Toussaint, J.P. (2004) Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, **85**, 2630-2637.
- Gracia, C., Burriel, J.A., Ibàñez, J.J., Mata, T., Vayreda, J. (2004) *Inventari Ecològic i Forestal de Catalunya. Mètodes*. Volum 9. CREAF, Bellaterra, 112 pp.
- Han, W.X., Fang, J.Y., Guo, D.L., Zhang, Y. (2005) Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytol*, **168**, 377-385.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965-1978.
- Johnson, N.C., Graham, J.H., Smith, F.A. (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, **135**, 575-586.
- Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463-1464.
- Mamolos, A.P., Elisseeu, G.K., Veresoglou, D.S. (1995) Depth of root activity of coexisting grassland species in relation to N and P additions, measured using nonradioactive tracers. *Journal of Ecology*, **83**, 643-652.
- Marschner, P., Timonen, S. (2005) Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. *Applied Soil Ecology*, **28**, 23-36.
- Oleksyn, J., Reich, P.B., Zytowski, R., Karolewski, P., Tjoelker, M.G. (2003) Nutrient conservation increases with latitude of origin in European *Pinus sylvestris* populations. *Oecologia*, **136**, 220-235.
- Paoli, G.D. (2006) Divergent leaf traits among congeneric tropical trees with contrasting habitat associations on Borneo. *Journal of Tropical Ecology*, **22**, 397-408.
- Paradis, E., Claude, J., Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289-290.
- Pärtel, M., Laanisto, L., Zobel, M. (2007) Constraining plant productivity-diversity relationships across latitude: The role of evolutionary history. *Ecology*, **88**, 1091-1097.
- Peñuelas, J., Sardans, J., Ogaya, R., Estiarte, M. (2008) Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: effect of simulated climate change. *Polish Journal of Ecology*, **56**, 613-622.
- Peñuelas, J., Sardans, J., Llusia, J., Owen, S., Carnicer, J., Giambelluca, T.W., Rezende, E.L., Waite, M., Niinemets, Ü. (2010) Faster returns on "leaf economics" and different biogeochemical niche in invasive compared with native plant species. *Global Change Biology*, **16**, 2171-2185.
- Pons, X. (2009) *MiraMon. Geographic Information System and Remote Sensing software*. Centre de Recerca Ecològica i Aplicacions Forestals, CREAF. Barcelona, Spain. ISBN: 84-931323-5-7.

- Powers, J.S., Tiffin, P. (2010) Plant functional type classifications in tropical dry forests in Costa Rica: leaf habit versus taxonomic approaches. *Functional Ecology*, **24**, 927-936.
- Raamsdonk, L.M., Teusink, B., Broadhurst, D., Zhang, N.S., Hayes, A., Walsh, M.C., Berden, J.A., Brudle, K.M., Kell, D.K., Rowland, J.J., Westerhoff, H.V., van Dam, K., Oliver, S.G. (2001) A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nature Biotechnology*, **19**, 45-50.
- R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Reich, P.B., Oleksyn, J. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Science USA*, **101**, 11001.
- Sardans, J., Rivas-Ubach, A., Peñuelas, J. (2011) Factors affecting nutrient concentration and stoichiometry of forest trees in Catalonia (NE Spain). *Forest Ecology and Management*, **262**, 2024-2034.
- Sardans, J., Rivas-Ubach, A., Peñuelas, J. (2012a) The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organism life style and ecosystem structure and function: a review. *Biogeochemistry*, **111**, 1-39.
- Sardans, J., Rivas-Ubach, A., Peñuelas, J. (2012b) The C:N:P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. *Perspectives in Plant Ecology Evolution and Systematics*, **14**, 33-47.
- Sardans, J., Peñuelas, J. (2013) Tree growth changes with climate and forest type are associated with relative allocation of nutrients, especially phosphorus, to leaves and wood. *Global Ecology and Biogeography*, **22**, 494-507.
- Sardans, J., Peñuelas, J. (2014a) Storage of the treasure nutrient, phosphorus, in forest wood. *Global Ecology and Biogeography* In press.
- Sardans, J., Peñuelas, J. (2014b) Climate, taxonomy and competition underlie different elemental concentrations and stoichiometries of forest species: the optimum "biogeochemical niche". *Plant Ecology*, **215**, 441-455.
- Sardans, J., Rivas-Ubach, A., Estiarte, M., Ogaya, R., Peñuelas, J. (2013) Field-simulated droughts affect elemental leaf stoichiometry in Mediterranean forests and shrublands. *Acta Oecologica*, **50**, 20-31.
- Simpson, D., Fagerli, H., Hellsten, S., Knulst, J.C., Westling, O. (2006) Comparison of modeled and monitored deposition fluxes of sulphur and nitrogen to ICP-forest sites in Europe. *Biogeosciences*, **3**, 337-355.
- Soil Survey Staff, 1998. Soil Taxonomy: A Basis System of Soil Classification for Making and Interpreting Soil Surveys. USDA Agric. Handb. Vol. 436. US Government Printing Office, Washington D.C.
- Stamova, B.S., Roessner, U., Suren, S., Laudencia-Chigcuano, D., Bacic, A., Beckles, D.M. (2009) Metabolic profiling of transgenic wheat over-expressing the high-molecular-weight Dx5 gluten subunit. *Metabolomics*, **5**, 239-252.
- Sterner, R.W., Elser, J.J. (2002) Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press.
- Stock, W.D., Verboom, G.A. (2012) Phylogenetic ecology of foliar N and P concentrations and N:P ratios across Mediterranean-type ecosystems. *Global Ecology and Biogeography* **21**, 1147-1156.
- Treseder, K.K., Vitousek, P.M. (2001) Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology*, **82**, 946-954.
- Villanueva, J.A., (ed). (2005) *Tercer Inventario Forestal Nacional (1997-2007)*. Ed. Ministerio de Medio Ambiente. Madrid.
- Webb, C.O., Donoghue, M.J. (2005) Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes*, **5**, 181-183.

- Webb, C.O., Ackerly, D.D. & Kembel, S.W. (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics*, **24**, 2098-2100.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, M., Garnier, J., Groom, E., Gulias, P.K., Hikosaka, J., Lamont, K., Lee, B.B., Lee, T., Lusk, W., Midgley, C., Navas, J.J., Niinemets, M.L., Oleksyn, Ü., Osada, J., Porter, N., Poot, H., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R. (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821-827.
- Yu, Q., Chen, Q., Elser, J.J., He, N., Wu, H., Zhang, G., Wu, J., Bai Y., Han, X.G. (2010) Linking stoichiometric homeostasis with ecosystem structure, functioning and stability. *Ecology Letters*, **13**, 1390-1399.
- Yu, Q., Elser, J.J., He, N., Wu, H., Chen, Q., Zhang, G., Han, X. (2011) Stoichiometry homeostasis of vascular plants in the inner Mongolia grassland. *Oecologia*, **166**, 1-10.

Figure captions

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

Figure 2. Plots of the PCA space cases (a) and variables (b), defined by the first two components of the PCA conducted with the foliar N, P, K, Ca and Mg concentrations 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 and the 5284 forest sites as cases and by the relationships between PC1 scores and phylogenetic position of the main taxonomic groups. The numbers within the PCA plot for cases correspond to the species. They are correlative in the phylogenetic tree. Arrows in this plot indicate the mean of the scores of the main families along the PC1 and PC2 components. Different letters on the arrows indicate significant differences ($P<0.05$). The colors of the arrows indicate the different families. The figure graphically shows the strong relationships between the phylogenetic distances and the differences in foliar composition among species and families. These relationships are shown in Figure 3. Means $\pm$ SE of the foliar N, P, K, Ca and Mg concentrations and N:P, N:K, P:K and Ca:Mg ratios of the various forest types. Different letters indicate significant differences ($P<0.05$).

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

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

Figure 6. Representation of the PCA space (cases and variables) defined by the two first PC axes with the foliar N, P, K, Ca and Mg concentrations and foliar 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 (a) and the non-Mediterranean forests as cases (b). All species separated by at least one of the first three axes (PC3 explained 14.9% of the total variance). The confidence intervals indicate Standard Errors for each species (n = from 5 to 1100).

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

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	
	K	P
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