Factors influencing the foliar elemental composition and stoichiometry in forest trees in Spain

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ABSTRACT

Concentrations of nutrient elements in organisms and in the abiotic environment are key factors influencing ecosystem structure and function. We studied how concentrations and stoichiometries of nitrogen (N), phosphorus (P) and potassium (K) in leaves of forest trees are related to phylogeny and to environmental factors (mean annual precipitation, mean annual temperature, forest type, and nitrogen deposition). Using data for 4691 forest plots from across Spain, we tested the following hypotheses: (i) that foliar stoichiometries of forest trees are strongly influenced by phylogeny, (ii) that climate, as an important driver of plant uptake and nutrient use efficiency, affects foliar stoichiometry, (iii) that long-term loads of N influence N, P and K concentrations and ratios in natural vegetation, and (iv) that sympatric species are differentiated according to their foliar stoichiometry, thereby reducing the intensity of resource competition.

Our analyses revealed that several factors contributed to interspecific variation in elemental composition and stoichiometry. These included phylogeny, forest type, climate, N deposition, and competitive neighborhood relationships (probably related to niche segregation effect).

These findings support the notion that foliar elemental composition reflects adaptation both to regional factors such as climate and to local factors such as competition with co-occurring species.

Keywords: Calcium; C:K; C:N; C:P; ecological stoichiometry; growth rate hypothesis; mean annual precipitation (MAP); mean annual temperature (MAT); Mediterranean ecosystems; magnesium; N:P; N:K; P:K; potassium; sulfur; water stress

Introduction

The leaf elemental composition and stoichiometry are important topics in forest research. Foliar N:P ratios have been shown to correlate with the growth rate of plants (Elser et al., 1996, 2000a and 2000b; Elser et al., 2010; Peñuelas et al., 2013a) and with the structure and function of plant communities (Peñuelas et al., 2013a; Sardans et al., 2012a; Sterner and Elser, 2002). Species with low foliar N:P ratios tend to grow rapidly and are more competitive for soil resources than species with higher N:P ratios, especially where nutrient availability is high (Elser et al., 2001 and 2003; Willby et al., 2001). In the last decade, there have been many studies of how foliar nutrient concentrations and stoichiometries (mainly N:P) vary along environmental gradients, both at regional (Santiago et al. 2004; Ordoñez et al., 2009; Sandel et al., 2010; Sardans et al., 2011) and global (Reich and Oleksyn, 2004) scales. Whereas some of these have found significant relationships between foliar N:P concentration ratios and climatic gradients (Kerkhoff et al., 2005; Reich and Oleksyn, 2004; Yuan and Chen, 2009), others have produced how contradictory results (Han et al., 2005; He et al. 2008; Lovelock et al., 2007;). Overall, these studies suggest that climatic conditions do affect foliar elemental stoichiometry, but that this relationship varies regionally and may be obscured by other factors such as the distribution of plant groups, soil type, and plant growth strategy (Sardans et al., 2011, 2012a). Since climate influences plant water availability, temperature and light intensity (Carte et al., 1997; Huxman et al., 2004; Voesenek and Pierik, 2008; Wang et al., 2012), climatic variables such as mean annual temperature (MAT) and mean annual precipitation (MAP) can be expected to determine, at least in part, the uptake of nutrients, and thus the foliar elemental concentrations and stoichiometry. However, these will also be influenced by other features of the ecosystem, including the dominant plant growth strategies (slow versus fast growth, shade versus light tolerance, etc.), community composition and soil nutrient heterogeneity. As a consequence, we can expect to observe a spectrum of foliar elemental composition and stoichiometry even at one site.

Most studies of plant stoichiometry have considered only N and P. However, potassium (K) is the most abundant nutrient in leaves after N, and considerably more abundant than P (Sardans et al., 2006b; Zheng and Shangguan, 2007). Together with N and P, K plays an important role in the physiology of terrestrial plants (Catmak, 2005). In particular, it controls the water content of leaves by controlling stomatal function, cell osmosis equilibrium and water fluxes (Babita et al., 2010; Fernández et al., 2006; Khosravifar et al., 2008; Laus et al., 2011; Milla et al., 2005; Oddo et al., 2011). Including potassium (K), therefore, should improve our understanding of the causes and consequences of plant stoichiometric shifts among species, natural gradients and competition conditions.

The concentration of K is especially significant in dry ecosystems because of its role in physiological responses of the plant to varying water availability and shifts in foliar N:P:Kratios are related to the change in response of plants to drought (Mairapetyan et al., 1999; Sardans and Peñuelas, 2007; Sardans et al., 2008b; Sardans and Peñuelas, 2008) in the Mediterranean forests and shrub lands. Moreover, some forests of the main biomes are K-limited (Nilsen and Abrahamsen, 2003; Olde Venterink et al., 2001; Tripler et al., 2004; Wright et al., 2011) and its concentrations and stoichiometrical relationships with N and P are important to control water use efficiency (WUE) in dry environments (Egilla et al., 2005; Graciano et al., 2005; Sardans et al., 2012a, 2012b; Welander and Ottosson, 2000). Some studies have found that a high concentration of K increases plant resistance to drought (Egilla et al., 2005; Stone and Moreira, 1996), and that stoichiometric relationships of K to N and P vary according to water availability (Peñuelas et al., 2013b; Rivas-Ubach et al., 2012; Sardans and Peñuelas, 2007; Sardans et al., 2011).

Other nutrients such as S, Mg or Ca may also limit growth in terrestrial plant communities (Baribault et al., 2012; Hailes et al., 1997; Lapeins et al., 2013; Naples and Fisk, 2010), albeit less commonly than N, P and K. Thus, in attempting to understand the factors that influence foliar elemental chemistry, there is a need for studies that also consider many more nutrients, including Ca, Mg and S.

Currently, apart from natural environmental circumstances such as climate conditions, the use of fertlilizer and atmospheric deposition of N are likely to trigger shifts in the N:P ratios of terrestrial plants (Peñuelas et al., 2012 and 2013a; Sardans et al., 2011 and 2012b; Veresoglou et al., 2014). Estimated loads of total annual atmospheric N deposition in Spain have approached 30 kg N ha^{-1} yr^{-1} (Avila and Rodà, 2012; Sanz et al., 2002). These levels are generally lower than those recorded in central Europe, but evidence from N concentrations of bryophytes in herbaria (Peñuelas and Filella, 2001) and a growing abundance of nitrophilous species in Spanish terrestrial ecosystems (Ariño et al. 2011) points to continuing N enrichment. In several sites of Europe, forest defoliation and dieback has been attributed to increasing N deposition (Jönsson et al., 2004; Pedersen and Bille-Hansen, 1995; Thomas et al., 2002), which has been shown to affect leaching of K, Ca, Mg and Na from soil in temperate and boreal areas of Europe and North America (Koptsik et al., 2007; Watmough et al., 2005). Meta-analysis of 107 independent studies of forest nitrogen (N) fertilization showed that the increase in N loads in temperate forest ecosystems decreased the concentrations of base cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) in soils and increased their concentrations in soil water and stream runoff (Lucas et al., 2011). Nutrient imbalance, particularly between N and other nutrients, is related to forest defoliation and dieback in European forests (Lomský et al., 2012; Veresoglou et al., 2014) and in other world areas (Wang et al., 2009). Under N deposition, plants are able to increase their P and K uptake capacity (Rowe et al., 2008). P limitation favors higher plant phosphorus uptake through enhanced activity of soil phosphatases (Tresseder and Vitousek, 2001) and root phosphatases (Gress et al., 2007; Phoenix et al., 2004) and by changing symbiotic fungi (Lilleskov et al. 2002). These mechanisms seem quantitatively insufficient to deliver enough phosphorus to alleviate P limitation (Vitousek et al., 2010), thus P becomes limiting (Turner et al. 2003; Bragazza et al. 2004) and P concentration decreases (Bragazza et al., 2004; Braun et al., 2010; Jonard et al. 2012; Lévy et al., 1999). Most studies show increase in N:P and N:K ratios under long-term N deposition in several ecosystem pools such as plants (Britton et al., 2008; Lawniczak et al., 2009; Thelin et al., 1998;) and soils (Olde Venterink et al., 2009) being N:K ratio related to changes in the plant species-specific composition and diversity of terrestrial ecosystems (Roem and Berendse, 2000). Thus, we would expect a wide effect of the gradients of N deposition on foliar elemental composition and stoichiometry of Spanish forests.

An understanding of how foliar elemental composition vary among tree species and along climatic gradients, and the different optima for elemental composition (not only N and P) among sympatric species, is relevant to forest management. These relationships should be considered in assessing the most promising species for reforestation and in managing existing forest areas. For example, some species may be more flexible than others in their stoichiometry, and better able to adapt to increasing N loads through deposition. An analysis of climatic and N-deposition data at the regional level can improve our understanding of the role of natural versus anthropogenic factors in determining plant stoichiometry and its imbalance. Correction of nutrient imbalances is a key issue for the success of afforestation, and survival and health of Spanish forests. Many of these are in arid areas where nutrient supply has a crucial influence upon the water-use efficiency of trees (Harvey and van den Driessche, 1999; Ripullone et al., 2004).

According to the biogeochemical niche hypothesis, coexisting plant species tend to use the main nutrients N, P and K (and other essential nutrients such as Ca, Mg and S) in differing proportions (Peñuelas et al., 2008 and 2010; Urbina et al., 2015), and therefore occupy distinct regions in the multivariate space generated by the concentrations and ratios of macro- and micronutrients in plant tissues. Since different plant structures and metabolic processes have distinct and divergent requirements for each of the essential nutrients, the species-specific biogeochemical niches should be the result of species specialization to particular abiotic conditions. Moreover, these niches should reflect the different species-specific strategies of growth and resources uptake and the differences in soil space and time occupation. Ultimately, these differences contribute to reducing direct competition among sympatric species (Peñuelas et al., 2008 and 2010; Urbina et al., 2015). Despite the existence of biogeochemical niches, however, it is also known that species exhibit varying degrees of stoichiometrical flexibility in response to environmental changes (including ontogenical and seasonal-climate changes) and competitive situations, probably under a tradeoff between flexibility and stability (homeostasis) capacity (Yu et al., 2010). Yu et al. (2011) observed that species with higher stoichiometry flexibility have higher concentrations of N and P and lower N:P ratios.

We investigated the factors influencing the stoichiometry of trees using existing data for Spanish forests. Our analysis was based upon four hypotheses. First, we predicted that the elemental stoichiometries of different forest species should be strongly determined genetically due to their long-term adaptation to specific abiotic and biotic environments with optimized metabolic and physiological functions and morphological structures that determine the specific use of various nutrients. Thus, distant taxonomic groups should have an overall different elemental composition and stoichiometry. Second, we hypothesized that an optimum stoichiometry for each climatic condition should be an important driver of plant uptake and nutrient use efficiency. These optima should reflect trade-offs among several functions such as plant growth, resource storage and/or anti-stress mechanisms for maximizing plant fitness in each of the particular climate situations. Thus, different sets of species growing in different forest types under different climatic conditions would tend to have different elemental composition and stoichiometry. Third, we hypothesized that in several European zones N, P and K concentrations and ratios in natural vegetation would reflect the long-term loads of N . Finally, we expected to find differentiation in foliar composition stoichiometry among sympatric species, as predicted in the biogeochemical niche hypothesis. These four hypotheses are not mutually independent. In fact, a phylogenetic signal in elemental composition and stoichiometry should be the result of long-term evolution under long-term climatic conditions and a determined neighbourhood environment. Besides testing these four hypotheses, we also tested the suitability of introducing in multivariate analyses other elements and ratios than N and P to test the biogeochemical niche hypothesis.

Methods

Foliar data

The data on foliar analyses were assembled from several sources, including: (i) the ecological forest inventory of Catalonia (Gracia et al., 2004), (ii) the third forest inventory of Spain (IFN3, Villaescusa and Díaz, 1998; Villanueva, 2005), (iii) the TRY database (Kattge et al., 2012); (iv) published papers available on the Web of Science till the year 2014; and (v) the International Co-operative Programme on Assessment on Monitoring of Air Pollution Effects on Forest (ICP Forest inventory) operated under the United Nations Economic Commission for Europe (UNECE) and the Convention on Long-range Transboundary Air Pollution (CLRTAP) (http://icp-forests.net/). All of these sources used comparable methods to determine foliar elemental concentrations. In all cases C and N concentrations were determined by the Kjeldahl or Carlo Erba chromatographic analyses, while the P and K concentrations were analyzed by atomic emission spectroscopy. The N:P:K concentration ratios, in leaves and needles, were calculated based on the mass. A total of 5039 data values of 4294 sites contributed to the data for estimation of N:P ratios and 3300 sites for foliar N:P:K ratios. To analyze all these data from different data sets we used the annual average information of the data from ICP Forest data set. Additionally, data for foliar concentrations of N, P, S, Mg and Ca were analyzed from a subset of 948 analyses corresponding to 381 plots from the ICP sampled in different years within the period 1990-2009. This forest inventory was randomly distributed across the Spanish forests. All foliar samples were collected during the period of 1990-2009, from fully expanded leaves. We used the N and S deposition (from EMEP) values estimated for each site and for the same year of foliar sampling.

We used data only from the plots with known geographical coordinates. All of the geo-referenced data were processed using the MiraMon 6.0 (Pons 2009). The species and forest types studied are mentioned in Table 1. The distribution of the plots and the dominant forest ecotypes are shown in Figure 1.

We also used soil N concentration data (soil Kjeldalh-N) of 53 Spain plots from the ICP Forest data base to analyze the effects of atmospheric N deposition on soil N concentrations in the organic soil layers.

Climatic data

For a comparative analysis, climatic data were obtained from two climatic models. For each stand of forest, we used MAT and MAP data derived from the WorldClim database (Hijmans et al., 2005) at a resolution of approximately 1 km^2 at the equator and from the Atlas climático digital de la Península Ibérica (Ninyerola et al., 2005) at a resolution of 4 ha. Both climatic models are based on interpolated values of climatic data provided by weather stations located around the territory and adjusted to the observed topography. MAP and MAT in both climatic models were calculated from long-term data (1950-1999 for the *Atlas climático* database and 1950-2000 for the WorldClim database). The results from the two models were almost identical with only minor improvements of one over the other depending on the model. We present in the results section the model that explained the most variance.

Atmospheric N deposition

Data for atmospheric N deposition 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 (Simpson et al., 2012) has been developed to estimate regional atmospheric dispersion and deposition of acidifying and eutrophicating compounds (N and S). This study used data for total atmospheric N and S deposition estimated annually for the period 1990-2009 with EMEP model rv3.8.1 over Europe using a grid size of 50 km × 50 km (EMEP, 2011). We used the N and S deposition values estimated for each site and for the same year of foliar sampling. Meteorological data were obtained from ECMWF-IFS Cycle36r1 (http://www.ecmwf.int/research/ifsdocs/); and the emissions during 2005 were derived from the data submitted to the UNECE CLRTAP in 2011 (EMEP 2011).

We also used data for N deposition estimated with the CHIMERE model of regional air quality. A detailed description of the model is available at the web link: http://www.lmd.polytechnique.fr/chimere/ and in the article by Menut et al. (2013). The simulations for the year 2005 were performed with the V200603par-rc1 version of CHIMERE at a horizontal resolution of 0.2º (approx. 20 km) nested to a European-scale simulation at a resolution of 0.5º, covering the Iberian Peninsula and the Balearic Islands. Further description regarding the set-up of the model can be obtained from Vivanco et al. (2009). The MM5 model was used to obtain meteorological fields (Reisner et al., 1998). Emissions were derived from the annual totals in the EMEP database (http://www.ceip.at/webdab-emission-database/emissions-as-used-in-emep-models). The spatial distribution of emissions was determined, and the speciation of non-methane volatile organic compounds was estimated as described by Vivanco et al. (2009). Oxidized and reduced nitrogenous compounds and dry and wet depositions were considered. The atmospheric N deposition values from CHIMERE are shown in Figure S1.

Statistical analyses

We tested the following specific hypotheses: (i) the phylogenetic signal of different foliar elemental concentrations and ratios individually, as well as the overall elemental composition and stoichiometry, using multivariate analysis, (ii) the role of mean annual precipitation (MAP), mean annual temperature (MAT), forest type (wet temperate deciduous angiosperms -WDA-, Mediterranean deciduous forests -WDF-, Mediterranean evergreen angiosperms -MEA-, wet temperate gymnosperms and alpine gymnosperms -WTG- and Mediterranean gymnosperms -MG- (iii) the role of N-deposition loads, and their possible interactions on foliar N, P and K concentrations and stoichiometries, and (iv) the differences in species-specific foliar elemental composition and stoichiometry among sympatric species.

The phylogenetic fingerprinting analysis of the foliar elemental composition was done by building a phylogenetic tree and obtaining the phylogenetic distances among species with the Phylomatic and Phylocom (Webb and Donoghue, 2005; Webb et al., 2008). Briefly, the Phylomatic uses a backbone plant mega-tree, which is primarily based on DNA data composed from a variety of studies to assemble a phylogenetic tree for the species of interest. Our phylogenetic hypothesis was based on the conservative mega-tree, 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 the R software (R Development Core Team, 2011) to check the phylogenetic signals among the foliar elemental composition of the species studied; thereby determining the extent to which the foliar N, P, K, Ca and Mg concentrations, the N:P, N:K, P:K, N:Ca, P:Ca, K:Ca, N:Mg, P:Mg, K:Mg and Ca:Mg ratios and the Principal Component Analysis (PCA) 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 variable tested. A value close to 1 indicates that most of the variability in the data can be explained by the phylogeny. We also correlated the phylogenetic distances between all pairwise comparisons of the most representative 34 forest species with the corresponding squared Mahalanobis distances from a discriminant function analysis (DFA) obtained with foliar N, P, K, Ca, Mg and S concentrations and N:P, N:K and K:P ratios.

To test our second and third hypotheses, we analyzed the effects of climate and N deposition on foliar elemental composition in European forest. The data were analyzed with general linear models (GLMs) using the foliar concentrations of the nutrients and the N:P:K concentration ratios, as dependent variables. Forest type (Mediterranean evergreen angiosperms, Mediterranean gymnosperms, Mediterranean deciduous forests, wet temperate and alpine gymnosperms and wet deciduous angiosperms) was used as a categorical independent variable, and climatic variables (MAP and MAT) and N deposition were used as continuous independent variables. The false discovery rate in the multiple correlations to correct the alpha inflation was used (García, 2004). The direct linear regressions of foliar concentrations of the nutrients and the N:P:K concentration ratios versus MAP, MAT and N deposition were conducted.

In the models to analyse the effects of MAP, MAT, and N deposition on needles, degree of spatial autocorrelation in the residuals was assessed using Moran's I correlograms and by plotting spatial maps of the distribution of residuals (following Carnicer et al., 2011; Stefanescu et al., 2011). To assess the level of significance at each distance class in the correlograms, 1000 permutations were computed using the resamp argument in the correlog function (ncf package) of the R package. On obtaining significant autocorrelation in the residuals in ordinary least squares (OLS) models, simultaneous autoregressive models (SAR) were applied spatially, to remove or at least decrease the spatial autocorrelation in the residuals of the models. In these models, we used the forest type as the categorical independent variable and MAP, MAT and the total atmospheric N deposition as the continuous independent variable. We used a factorial design, but in the final models, we maintained the interactions only among the significant independent variables. To detect the overall relationship (direct and indirect) of climate variables and N deposition on foliar N, P and N:P ratios, we performed Structural Equation Modelling (SEM). We fitted the different models using the sem R package (Fox et al., 2013) and achieved the minimum adequate model using the Akaike information criterion (AIC). Standard errors and the significance level (*P*-value) of the direct, indirect and total effects were calculated using the bootstrap (with 1200 repetitions) technique (Davison et al., 1986; Mitchell-Olds, 1986).

To determine whether the nutrient concentrations and foliar N:P:K concentration ratios could discriminate among the forest types, we performed PCAs. We also conducted a DFA in the subset of data from the ICP forest inventory of 381 plots for which we also had the S, Mg and Ca foliar concentration data, for a homogenous dataset randomly distributed across the Spanish forested areas. Both ordination analyses are complementary (Elliot et al., 2007; Johnson et al., 2003; Qadir et al., 2008; Stamova et al., 2009). The DFA is a supervised statistical algorithm that establishes an optimal separation between groups by maximizing the between-group variance and minimizing the within group variances (Raamsdonk et al., 2001), whereas the PCA does not maximize between-groups variation against within-group variance. For this subset of plots, we conducted the PCA analysis with foliar N, P, K, S, Ca and Mg concentrations and N:P, N:K and P:K ratios as a proxy of biogeochemical niche of species, to determine the significance of any effect of the climate and N deposition on the biogeochemical niche of species.

To test our fourth hypothesis, that the species growing in similar climatic conditions should also present different elemental composition and stoichiometry within the group of typical Mediterranean species (Mediterranean evergreen angiosperms, Mediterranean gymnosperms and Mediterranean deciduous forests), we performed the PCA and the FDA analyses. The analyses were done using the above variables to study the degree of segregation of biogeochemical niches among species of the same climatic area or forest type that frequently compete. The squared Mahalanobis distances from the discriminate functional analysis among different species were used. We also used one-way analyses of variance to detect any differences among the PC scores of the first and second components for the three forest types. Regressions of the PC scores of the first and second components with the climatic variables and N deposition levels were conducted for detection of possible relationships of biogeochemical niche with climatic variables and N deposition. The variables were log-transformed for normalization of their distributions. We also determined the species overlapping frequency (coincidence in the same community) (0 = no overlapping, 1 = occasionally overlapping, 2 = moderate overlapping, 3 = frequent overlapping), in the set of the 13 most representative Mediterranean species, based in Rivas-Martinez et al. (2001) De Bolos and Vigo (2001), CREAF, Catalonia cover map, (2013) and Folk et al. (1984). Subsequently, the frequency of overlapping was correlated with the scores of the first PC axes obtained by using foliar log-transformed foliar N, P, K, Ca, Mg and S concentrations and log-transformed foliar N:P, N:K and P:K ratios as variables. The analyses were performed using StatView 5.0.1 (SAS Institute Inc., Berkeley Ca, USA), Statistica 6.0 (StatSoft, Inc. Tule, Oklahoma, USA) and R (Core development team, 2011).

Results

Phylogenetic signal of foliar elemental composition and stoichiometry

Phylogenetic signals were observed for foliar N, K, Ca and S concentrations (Table 2). In the case of P concentration, the phylogenetic signal was marginally significant, whereas it was completely absent in case of foliar Mg concentration (Table 2). We found no phylogenetic signal for foliar N:P and N:K ratios, and a marginally significant one for foliar P:K ratio (Table 2). In a principal components analysis of foliar data for 37 tree species of Spanish forests (N, P, K, Ca, Mg and S concentrations and log-transformed N:P, N:K and P:K ratios), we found significant phylogenetic signals in the PC1, PC3 and PC4 axes and a marginally significant signal in the PC2 axis ($P=0.07$) (Table 2). In addition, pairwise differences between species in PC1, PC2 and PC3 scores were significantly correlated with differences in phylogenetic distances (Figure 2). Foliar N:P ratios of gymnosperms were significantly lower than those of angiosperms (Figure 3).

Climate and distribution of forest type

The two climatic models provided identical climatic data (data not shown). Figure S2 shows the distribution of MAT and MAP across Spain. Despite variation due to altitudinal and continental effects, the data reveal a clear gradient of increasing MAP and decreasing MAT from southeast to northwest, reflecting the transition from Mediterranean to temperate Atlantic climates. This climatic gradient is associated with changes in tree species, from typical Mediterranean species (Mediterranean evergreens angiosperms and

Mediterranean gymnosperms) at the sites of higher MATs and lower MAPs to non-Mediterranean species (wet temperate deciduous angiosperms and wet temperate and alpine gymnosperms) at the sites of slightly lower MATs and higher MAPs, with Mediterranean deciduous forests occupying intermediate sites (Figure 4).

Effects of climate, forest type and N deposition on foliar elemental composition

Data from general databases (ICP forest, TRY and Catalan and Spanish forest inventories). MAP was positively correlated with the foliar N and P concentrations and negatively with the N:P ratios (Table 3), whereas MAT was negatively correlated with the foliar P concentrations and positively with the N concentrations and the N:P ratios (Table 3). The deciduous forests generally had the highest foliar elemental concentrations, and the Mediterranean gymnosperms had the lowest (Figure 5). Wet deciduous angiosperm and Mediterranean gymnosperm forests had the highest foliar N:K ratios, while the wet temperate and alpine gymnosperms had the highest foliar P:K ratios (Figure 3).

The geographical distributions of the available values of foliar N and P concentrations and N:P ratios are shown in Figure S3. The log-transformed foliar N:P ratios were negatively correlated with log-transformed P concentrations (*R*=0.61, *P*<0.0001), indicating a significant trend of decreasing foliar N:P ratios with increasing P concentrations. When both foliar N and P concentrations increased, P tended to increase more than N, with the consequence that nutrient rich leaves tended to have lower N:P ratios (R=0.61, *P*<0.0001) (Figure 6). This effect was observed in all forest types. The PCA analysis with log-transformed foliar N, P and K concentrations and N:P, N:K and P:K ratios separated the five forest types along the first two axes (Figure S4). The angiosperm and gymnosperm forests separated along PC1 (explaining 42.3% of the total variance), with foliar K concentrations and foliar N:K and P:K ratios as the dominant factors, separated, whereas Mediterranean forests from wet deciduous angiosperms separated along PC2 (explaining 31.6% of the total variance), with foliar N and P concentrations as the main factors (Figure S4). The results also highlight the negative correlation between the foliar N:P ratios and N and P concentrations, with the slow-growing evergreens placed toward lower foliar N and P concentrations and higher N:P ratios (Figure S4).

In the general linear models (GLM) with climatic variables (MAT and MAP), forest type, and N deposition as independent variables (Table S1), identical results (data not shown) were obtained for different climatic and N-deposition models. N deposition had a positive interaction with MAP and MAT, increasing the foliar N concentration. Atmospheric N deposition thus increased foliar N concentrations only when accompanied by high values of MAP and/or MAT (Table S1). N deposition decreased the P concentrations, thus significantly increasing foliar N:P ratios (Table S1). The direct regression between log-transformed foliar N concentration and log-transformed N deposition (without taking into account the climate variables) was also significant (Figure 7). Atmospheric N deposition was negatively correlated with foliar P and K concentrations and positively correlated with foliar N:P ratio (Figure 7). Despite different species having distinct elemental profiles, some species were relatively variable in their elemental composition (Figure S5). Among the four most representative species, we observed that the foliar P concentration decreased with MAP and MAT only in *P. nigra* (Figure S5).

Data from ICP forest database. To further study the possible effects of climate and N deposition on foliar chemical composition, we used data for 381 forest plots from the ICP Forest database. The results from the ordinary least square models and from SAR were identical (Table S2). MAT and MAP, forest type and N deposition together explained 77%

of the variance in foliar N concentrations and 38, 54 and 43% of the variability in P and K concentrations and N:P ratios, respectively (Table S2). Autocorrelation and ordinary least square models produced similar results (Table S2). Foliar N concentrations were considerably higher at sites where high atmospheric N deposition coincided with elevated MAP and, to a lesser extent, MAT. However, high atmospheric N deposition reduced the foliar P concentrations, thereby significantly increasing the N:P ratios (Table S2). Consistent with these results, we observed that log-transformed total N deposition was positively related with soil Kjeldahl-N across 53 plots from ICP database (Figure 8). The PCA conducted with log-transformed foliar N, P, K, Ca, S and Mg concentrations and N:P, N:K and P:K ratios distinguished deciduous, evergreen and gymnosperm species mainly along PC1, which was essentially loaded with foliar N and S concentrations (Figure S6). The score coefficients of PC1 were negatively correlated with MAP and N deposition (Figure S6). Although these relationships were weak, they explained a part of the variability in overall foliar elemental composition and stoichiometry, signifying that higher foliar N concentrations are related to higher levels of MAP and N deposition. Most pairwise species comparisons of the DFA detected statistical differences between the species' elemental compositions (Table 4). The structural-path analysis model with climate (MAP and MAT) and taxonomy (angiosperms versus gymnosperms) as exogenous variables and N deposition, foliar N and P concentrations and foliar N:P ratio as endogenous variables, provided an overview of the relationships among these variables (Figure 9) and of the total, direct and indirect effects (Figure 10). The model explained 47 and 88% of total variance of foliar N concentration and N:P ratio, respectively (Figure 9). Notably, there was a considerable positive relationship between MAP and N deposition, a direct positive relationship between N deposition and foliar N concentration and an indirect relationship of N deposition and foliar N:P ratio resulting from an indirect effect through foliar N concentration (Figures 9 and 10). There was a negative relationship of MAT with foliar N concentration and even more with foliar P concentration, and hence a negative total relationship of MAT with foliar N:P ratio (Figures 9 and 10). In contrast, there were positive relationships between MAP and foliar P concentration and with foliar N concentration (the latter reflecting in part an indirect effect of N deposition), and therefore also in a positive relationship between MAP and foliar N:P ratio (Figure 10).

Mediterranean species and their differences in elemental composition and stoichiometry

In the PCA, conducted with the same variables but only for a subset of typical Mediterranean forests (n=306), most species tended to separate throughout the space formed by the first two PCA axes (Figure 11), showing that these species of the same Mediterranean group, which frequently coexisted and competed, tended to have different foliar elemental compositions. Moreover, PC2 correlated significantly with N deposition (higher N deposition related to higher foliar N concentrations and N:P ratios), although the relationship only explained 5% of the variation (Figure 11). Pairwise species differences in the PC1 scores increased with increasing overlapping presence of the other species in the same community (Figure 12). In the corresponding DFA analysis, the 13 dominant Mediterranean species of Spain each had a distinctive profile in terms of foliar elemental composition and stoichiometry (Table 5).

Discussion

Phylogeny, leaf elemental composition and stoichiometry

The results indicate that leaf elemental composition exhibits a strong phylogenetic signal for five of the six elements analyzed and in terms of stoichiometry. Thus, our first hypothesis - that the foliar elemental composition is related with long-term evolution and natural selection processes - is supported.

Some of these phylogenetic differences may be of great ecological relevance. For example, in all forest types lower foliar N:P ratios tend to be associated with higher N and P concentrations, suggesting that N:P ratios tend to decrease in nutrient-rich environments. Such relationships are mostly observed in angiosperms and not in gymnosperms, which mainly had lower foliar N and P concentrations and lower N:P ratios. The differences between angiosperms and gymnosperms explain a significant part of the variation in foliar elemental composition and stoichiometry among species, and strongly suggest that evolutionary processes have played an important role. Alternatively and/or complementarily to this hypothesis, at least in these Spanish forests, where gymnosperms are usually the initial successional species, the pioneering success of gymnosperms could also account for the low foliar N:P ratios due to the higher proportional availability of P relative to N in the initial phases of succession, for example, after a fire event. The latter hypothesis seems reasonable for our dataset, because the most abundant gymnosperm forests, such as those dominated by *Pinus halepensis*, *P. pinaster*, *P. nigra,* and *P. sylvestris* usually constitute the early forest stage, and are most frequently replaced by angiosperms, such as *Quercus ilex*, *Q. suber*, *Q. humilis*, *Q. petrea*, *Q. pyrenaica,* and *Fagus sylvatica* in more advanced successional stages. Further, our results suggest that coniferous trees might grow better in soils with lower N:P ratios because their optimum mass-based N:P ratio is under 14, which could indicate high P requirements (Bott et al., 2008; Güsewell and Bollens, 2003). In contrast, angiosperms with mass-based N:P ratios above 15-16, which enable the trees to grow better in soils with high N:P ratios, are frequently associated with the older soils of more advanced successional stages.

Relationships of climate and forest type with foliar N and P concentrations and N:P ratios

The present work supports previous studies carried out to analyze the effects of climate on foliar N and P concentrations and N:P ratios. Optimum climatic conditions for growth often coincide with high foliar N and P concentrations and with low N:P ratios (Kerkhoff et al., 2005; Reich and Oleksyn, 2004; Sardans and Peñuelas, 2013; Yuan and Chen, 2009). The positive effect of water availability on mineralization and nutrient availability can be especially important in naturally dry environments, such as the Mediterranean ecosystems (Jarvis et al. 2007; Sardans and Peñuelas 2005 and 2010; Sardans et al. 2008c). We have observed that MAP, the climatic factor that most frequently limits growth in the forests of Spain, is positively correlated with higher foliar N, P and K concentrations. On the other hand, relationships with MAT are weaker and opposite to those of MAP. The multifactorial GLM models indicated that the positive relationship between MAP and foliar N, P and K concentrations occurred particularly where high MAPs coincided with high MATs and N depositions. Further, these relationships were partially due to the effects of climate on the distribution of different forest types, with deciduous forests occupying the wettest environments and having the highest foliar N, P and K concentrations. Deciduous angiosperms in Spain are frequently associated with optimum climatic conditions (as shown in Figure 2). It has been noticed that in the PCA analysis, foliar K concentration is also higher for the forests occupying the wettest environments and the Mediterranean evergreen angiosperms. This indicates the dual importance of K being the main element loading in the PC1, discriminating angiosperms from gymnosperms and also separating forests occupying the wettest environments from other forests. The higher growth capacities of wet deciduous angiosperm forests in Catalonia had already been associated with higher water availability and foliar N, P and K concentrations (Sardans and Peñuelas, 2013). The use of precipitation and temperature distribution during the growth period instead of MAP and MAT would very likely yield closer relationships between climate and elemental composition, but these data were not available. Extreme climatic events could also be even more strongly related to plant elemental composition (Kreyling et al., 2010; Urbina et al. 2015). Moreover, although each species tends to have a specific foliar elemental composition, we detected certain degree of flexibility in species-specific elemental composition along the climate gradients. Thus, another significant part of the variance of the foliar elemental composition and stoichiometry among different species and also among individuals of the same species can be explained by the prevailing climatic conditions, as our second hypothesis claimed.

N deposition and forest stoichiometry

The large gradients of N deposition across the forested areas of Spain allowed us to detect the influence of deposition on forest elemental composition. All analyses indicated that N deposition had already affected some Spanish forests, particularly, due to an increase in the foliar N concentration and N:P ratios. Atmospheric N deposition increased foliar N concentrations, particularly in areas where high levels of N deposition coincided with high levels of MAT and, mainly, of MAP. This effect suggests that, under climatic conditions favourable for growth, high N loadings tend to be retained by forests. If this trend continues, however, the species with high foliar N:P ratios could be favoured under high levels of N deposition. The results also suggest that the increase in P-limitation under high levels of N deposition could benefit angiosperms over gymnosperms in Spanish forests, especially in areas where the climate favours rapid tree growth. The data coincided with the decrease of gymnosperms forest cover in favor of angiosperms forest cover in Spanish forests (Carnicer et al., 2013; Coll et al., 2011).

Several previous studies have detected increases in foliar N concentrations in terrestrial plants in Europe owing to N deposition (Dukesnay et al., 2000; García-Palacios et al., 2012; Kang et al., 2011; Solberg et al., 2002) and have also detected increases in N:P ratios (Dukesnay et al., 2000; Kang et al., 2011), together with decreases in foliar P concentration related to enhancement of P defficiences (de Vries et al., 1995; Dukesnay et al., 2000; Erisman et al., 1998; Jonard et al., 2015; Solberg et al., 2002). Our results, however, show that foliar P concentrations can decrease as a consequence of N deposition and that a general change in foliar elemental composition involving other elements such as K could also occur on a global scale due to uninterrupted N loadings from N deposition. The moderate direct effects of N deposition on foliar N concentrations that we observed were consistent with studies performed in undisturbed mountainous areas of North Spain (Amores et al., 2006). Long-term studies in Northeast Spain, among the regions with highest levels of atmospheric N deposition, performed in the undisturbed catchments showed that these ecosystems are still far from N saturation, because most of the deposited N is retained within the ecosystem, and only small increase in NO_3^- concentrations could be detected in stream water (Àvila and Rodá, 2012). Indeed, in a previous study, we have found a positive relation between N deposition and soil organic-N for 53 forest plots, suggesting that those soils had a considerable capacity to retain N.

Increase in N deposition can increase the immobilization of P in the soils of forested areas through the high uptake of P by the soil food web (Piatek et al., 2009), and several studies have concluded that high and continuous N loadings increase P limitation (Boxman et al., 1994; Gradowski and Thomas, 2008; Huang et al., 2012 Phoenix et al., 2003). However, when the N loadings increase, plants and soil organisms' enhance the synthesis of their phosphatases to increase P uptake. Such mechanisms do not prevent a lasting increase in N:P ratios (Peñuelas et al., 2013a). Regarding our third hypothesis, although there are no adequate data of temporal series of N deposition versus foliar composition, there is some evidence that current high N deposition loads in certain areas of Spain may lead to changes in foliar elemental composition, especially in the more productive areas.

The case of sympatric species

Different tree species growing in the same Mediterranean climate tend to have contrasting foliar elemental profiles. Such contrasts were especially clear among species that habitually coexist, such as *Q. ilex*, *P. halepensis* and *Juniperus oxycedrus*, or *Q. suber*, *P. pinea* and *P. pinaster*, and tended to be less apparent or not significant among species that are rarely found together, such as *P. pinaster* and *P. nigra* (CREAF, Catalonia cover map, 2013). Thus, these results support previous studies that found a tendency of coexisting plant species to have different elemental compositions (Peñuelas et al., 2008, 2010). This dimension of biogeochemical niche would coincide with the complementary niche hypothesis (Hector et al., 1999). Neighbouring species may exhibit different interactions with the environment due to their species-specific functioning and morphology. Indeed, coexisting plant species might even differentiate their stoichiometries to avoid direct competition by enhancing diversity in their uses of nutrients or could also change their stoichiometries by shifting some functions to better cope with different competitive circumstances. These strategies should help reduce the negative impacts of interspecific competition and also improve the productive capacity of the global community, as expected by the complementary niche hypothesis (Hector et al., 1999).

The combined effects of climate, forest type and competition on overall foliar composition: testing the biogeochemical niche hypothesis (BNH)

The biogeochemical niche of each species, understood as the region occupied in the multivariate space generated by the content of macro- and micronutrients in plant tissues (Peñuelas et al., 2008, 2010), was significantly different among the forest species of different climatic areas (Mediterranean forests relative to wet temperate and alpine forests) and between different taxonomic groups (angiosperms and gymnosperms). Moreover, the results showed that sets of plant species growing under the same climatic conditions that frequently coexist tend to diverge in their elemental composition. The results thus showed that apart from the shift of foliar composition in coexisting species, taxonomy, current climatic conditions, and human driven impacts such as atmospheric N deposition also determined a significant and important part of the variability in foliar elemental composition and stoichiometry. In this context, we have observed that foliar compositions and stoichiometries were more similar among the Mediterranean evergreen angiosperms than other angiosperms growing under different climatic conditions. For example, the evergreen *Q. suber*, typical of acidic soils, has a foliar elemental composition more similar to that of the evergreen *Q. ilex*, which frequently prefers neutral and basic soils, than to that of other *Quercus* species growing in acidic soils but in wetter climates, such as *Q. pyrenaica*, *Q. petraea* or *Q. robur* (Figure 6). Similarly, gymnosperms such as *P. pinaster* growing in acidic soils have foliar elemental compositions more similar to those of other gymnosperms such as *P. halepensis* growing in basic soils than to those of angiosperms growing in acidic soils but in the same climate, such as *Q. suber*, or in a different climate, such as *Q. robur* (Figures 6 and 8). Species thus tend to occupy the climate areas where they are better adapted and this explains a part of their foliar composition. The overall results were thus consistent with the BNH.

Climate is not the only driver of the evolutionary processes; factors such as interspecific competition and soil nutrient status are also important. The results were thus consistent with the idea that each individual species, being the original product of a singular evolutionary history under specific abiotic (climate, soil type, etc.) and biotic (trophic relationships, neighbor competitors, etc.) conditions, tends to have it own elemental profile and stoichiometry. Nonetheless, as observed here, species may exhibit flexibility in stoichiometry in response to environmental changes (including ontogenical and seasonal changes) and competitive conditions (Dybzynski et al., 2013). There is a tradeoff between adaptation capacity (flexibility by short term responses) and stability (some degree of homeostasis obtained under species-specific evolutionary history) (Yu et al., 2010). These two components can also differ for the evolutionary process of a particular species. Moreover, as predicted by the BNH, the concentrations of elements such as K and Ca had differences similar to those of P and N among species of different climatic areas, forest types or taxonomic groups and among species growing within a climatic area and forest type. These other elements thus warrant more attention in ecological stoichiometric studies in terrestrial plant communities.

Foliar composition and forest management

A knowledge of the elemental profiles (not only N and P) of sympatric species at a regional scale could be useful in deciding which species or species combinations to plant at particular sites, both for forest conservation and wood production. The sites with high N deposition that coincides with more productive climatic conditions tend to present greater nutrient imbalances. In this regard the fact that N deposition generates larger foliar P decreases in gymnosperms than in angiosperms could also be underlying in a higher decrease of gymnosperm forest cover in favor of angiosperm forest cover observed in Spain forests (Carnicer et al., 2013; Coll et al., 2013). Coll et al. (2013) correlated this change in coverage of the two clades to climate change effects, but the results of this study suggest that the impacts of N deposition can also contribute to this shift. This factor should be considered in new reforestation politics. The forest species with natural foliar composition with high N and low concentrations of other elements would be most adequate in these conditions, not only due to their better survival but also because they might improve the system imbalance. As commented previously, successful afforestation and maintaining healthy forests may depend upon correcting nutrient imbalances, especially in more arid regions where water-use efficiency is critical. Thus, the information of the natural elemental composition, including the concentration of other elements than N and P involved in water use, is necessary for afforestation programs, including the possibility for soil amendments. Forest management has been very focused on N and P, and yet this study shows that other elements such as K or Ca can have different optima among species in a range even greater than that of N and P. This fact should be taken into consideration in forest management. This study provides data on nutrient availability in forest under different climatic regimes that will be useful when taking decisions for the management of Spanish forests.

Conclusions and final remarks

1. Differences among species of the foliar concentrations of five of the six elements analyzed and also overall foliar composition and stoichiometry were related to the corresponding phylogenetic distances among species. When comparing phylogenetic groups we further confirmed this link. For example, gymnosperms had lower foliar N and P concentrations and N:P ratios than did angiosperms. Our results showed that phylogenetic distances explain a significant part of overall species-specific elemental foliar composition and stoichiometry differences.

2. Climate and forest type accounted for a significant part of the variance in foliar N and P concentrations and N:P ratios among different forest tree species, and explained a significant part of overall species-specific elemental foliar composition and stoichiometry differences. Foliar N and P concentrations generally increased as N:P ratios decreased, and sites with better climatic conditions for plant production had lower N:P ratios. All these results showed that each climate condition is an important driver fot plant nutrient uptake and use.

3. N deposition tended to increase foliar N concentrations and N:P ratios, and to decrease foliar P concentrations, particularly when coinciding with high MAPs and MATs. Thus, N deposition had a weak but significant effect explaining part of the overall variation in elemental foliar composition and stoichiometry.

4. Sympatric species have different elemental compositions. Species of the same Genus tend to be more different in elemental composition when they are sympatric than allopatric. Thus, the data are consistent with the niche segregation hypothesis: that coexisting plant species avoid direct competition by having contrasting stoichiometries.

5. The overall results supported the hypothesis that foliar elemental composition and stoichiometry (here represented by N, P, K, Ca, Mg and S) are species-specific supporting

the biogeochemical niche hypothesis. This study showed that the species-specific foliar elemental composition and stoichiometry are, to a large extent, explained by taxonomical differences, current climatic conditions, and competitive neighborhood relationships (probably related to niche segregation effect). Human-driven N deposition can also exert some influence explaining a minor but significant part of overall leaf composition and stoichiometry variability among the studied set of forest species in Spain.

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Figure captions

Figure 1. Distribution of the forests showing the most representative forest type of each.

Figure 2. Biplots representing (A) the PC1 and PC2 axes and (B) the PC1 and PC3 axes of the Principal Component Analysis conducted with log transformed foliar N, P, K, Ca, Mg and S concentrations and log transformed foliar N:P, N:K and P:K ratios of the main 37 Spain's forest species*.* The relationships of all pair-wise species differences in the scores of PC1 (C), PC2 (D) and PC3 (E) with the corresponding pair-wise species phylogenetic distances*.*

Figure 3. Mean ± SE of foliar N:P, N:K and P:K ratios in the various forest types (evergreens, Mediterranean gymnosperms, non-Mediterranean gymnosperms and deciduous forests). Different letters indicate significant differences ($P<0.05$).

Figure 4. Distribution of the forest tree species as a function of the mean annual temperature (MAT) and mean annual precipitation (MAP). Large color symbols indicate the means of MAT and MAP for each forest type.

Figure 5. Mean ± SE of foliar N, P and K concentrations in the various forest types (Mediterranean evergreen angiosperms, Mediterranean gymnosperms, Mediterranean deciduous forests, wet temperate and alpine gymnosperms and wet temperate deciduous angiosperms). Different letters indicate significant differences ($P<0.05$).

Figure 6. Relationship of log foliar N:P ratios with log P concentrations in 4691 forest plots.

Figure 7. Relationships of (A) log foliar N concentration (%, DW), (B) log foliar P concentration (%, DW), (C) foliar N:P ratio and (D) log foliar K concentration (%, DW) with log N deposition (mg m^{-2} y^{-1}).

Figure 8. Relationships between log soil N-Kejdahl concentration and log N deposition across 53 plots of Spain (ICP forest data).

Figure 9. Structural-path model with climate (MAT and MAP) and taxonomy (angiosperms versus gymnosperms) as exogenous variables and N deposition, foliar N and P concentrations and foliar N:P ratio as endogenous variables. Number next to each arrow indicates the Pearson R coefficient between the two corresponding variables and the number below it (between brackets) indicates the corresponding level of significance (P-value).

Figure 10. Total, direct and indirect effects of N deposition (N dep), MAP, MAT, foliar N concentration (foliar [N]), foliar P concentrations (foliar [P]) and (angiosperms versus gymnosperms, converted in a dummy variable, 0 = angiosperms, 1 = gymnosperms) on foliar N concentration, foliar P concentrations and foliar N:P ratio. Data obtained by using the bootstrap (with 1200 repetitions) technique (Davison et al., 1986; Mitchell-Olds, 1986).

Figure 11. Loadings (A) and scores (B) of the PCA conducted using log foliar N, P, K, Ca, S and Mg concentrations and N:P, N:K and P:K ratios as variables and the data from 260 plots from the ICP forest dataset with the 13 most representative Mediterranean tree species of Spanish forests. The mean values and corresponding SEs of the PC1 and PC2 scores for each forest type are presented in panel (B). Statistically significant differences in scores among species are indicated by different letters along the corresponding axes. (C) Relationship of PC2 scores with log N deposition.

Figure 12. Relationships of the differences in PC1 scores (obtained by using log transformed foliar N, P, K, Ca, Mg and S concentrations and log transformed foliar N:P, N:K and P:K ratios as variables) between pairwise species and the corresponding pairwise species overlapping frequency (coincident in the same community) (0 = no overlapping, 1 = occasionally overlapping, 2 = moderate overlapping, 3 = frequent overlapping) for the 13 most representative Mediterranean species (Rivas-Martinez et al., 2001; De Bolos and Vigo 2001; CREAF, Catalonia cover map, 2013; and Folk et al. 1984).

Table 1. Species included in the study.

Forest tree species
Mediterranean evergreen angiosperms *Arbutus unedo*, *Eucalyptus* sp., *Ilex aquifolium*, *Olea europea*, *Quercus ilex*, *Quercus suber*.
Mediterranean deciduous angiosperms *Castanea sativa*, *Quercus canariensis*, *Quercus canariensis* x *humilis*, *Quercus cerrioides, Quercus cerrioides* x *humilis*, *Quercus faginea*, *Quercus pyrenaica*.
Wet temperate deciduous angiosperms *Betula pendula*, *Fagus sylvatica*, *Fraxinus angustifolia*, *Fraxinus excelsior*, *Platanus hybrides*, *Populus nigra*, *Populus tremula*, *Prunus malaheb*, *Quercus humilis*, *Quercus petraea*, *Quercus robur*.
Mediterranean gymnosperms *Juniperus oxycedrus*, *Juniperus phoenica*, *Juniperus thurifera*, *Pinus halepensis*, *Pinus nigra*, *Pinus pinaster*, *Pinus pinea*.
Wet temperate and alpine gymnosperms *Abies alba*, *Cedrus deodorata*, *Pinus radiata, Pinus sylvestris, Pinus uncinata, Pseudotsuga menziesi*.

Table 2. 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 log transformed foliar elemental compositions (N, P, K, Ca, Mg and S concentrations and log transformed foliar N:P, N:K and P:K ratios as variables) of the most representative 37 species of Spanish forests. The variables with significant (P<0.05) phylogenetic signals are highlighted in bold type (K=statistic, P=level of signification).

Variable	Phylogenetic signal statistics	
	K	*P*
Foliar [N]	**0.171**	**0.001**
Foliar [P]	0.114	0.065
Foliar [K]	**0.182**	**0.006**
Foliar [Ca]	**0.204**	**0.002**
Foliar [Mg]	0.105	0.25
Foliar [S]	**0.216**	**0.004**
Foliar N:P ratio	0.135	0.31
Foliar N:K ratio	0.085	0.73
Foliar P:K ratio	0.174	0.09
PC1	**0.170**	**0.04**
PC2	0.117	0.074
PC3	**0.256**	**0.002**
PC4	**0.165**	**0.002**
PC5	0.144	0.11
PC6	0.093	0.60

Table 3. Coefficients of correlation (*R*) and levels of significance (*P*) of the correlations of foliar N and P concentrations and N:P ratios with MAP and MAT. All variables were log-transformed. Bold type indicates statistically significant correlations at *P*<0.01.

Climatic data		**log foliar N concentration**	**log foliar P concentration**	**log foliar N:P ratio**
Data from WorldClim database	**log MAP**	**_R_=0.14** **_P_<0.001**	**_R_=0.24** **_P_<0.0001**	**_R_=-0.13** **_P_<0.001**
	log MAT	**_R_=0.40** **_P_=0.007**	**_R_=-0.17** **_P_<0.0001**	**_R_=0.24** **_P_<0.0001**
Data from Atlas Climatico digital de España	**log MAP**	**_R_=0.29** **_P_<0.001**	**_R_=0.28** **_P_<0.001**	**_R_=-0.045** **_P_=0.002**
	log MAT	*R*=0.029 *P*=0.051	**_R_=-0.18** **_P_<0.0001**	**_R_=0.23** **_P_<0.0001**

Table 4. Test statistics for squared Mahalanobis distances between all pairwise comparisons of tree species from the discriminate functional analysis with log-transformed foliar concentrations of N, P, K, S, Ca and Mg and log-transformed N:P, N:K and P:K concentration ratios as variables using the 375 plots from the ICP data set dominated by the 21 most representative species of forest trees as cases. Significant pairwise distances are highlighted in bold type.

	B. pendula	***C. sativa***	***Eucalyptus sp***	***F. sylvatica***	***J. oxycedrus***	***J. thurifera***	***O. europea***	***P. halepensis***	***P. nigra***	***P. pinaster***	***P. pinea***	***P. sylvestris***	***P. uncinata***	***Q. faginea***	***Q. humilis***	***Q. ilex***	***Q. petraea***	***Q. pyrenaica***	***Q. robur***	***Q. suber***
A. alba	**41.5 *P*<0.001**	**50.6 *P*<0.001**	**15.1 *P*<0.001**	**33.1 *P*<0.001**	10.8 *P*=0.16	6.11 *P*=0.23	**12.2 *P*=0.04**	**43.0 *P*<0.001**	**35.5 *P*<0.001**	**65.1 *P*<0.001**	**74.3 *P*<0.001**	**32.0 *P*<0.001**	**45.5 *P*<0.001**	**27.5 *P*<0.001**	**25.4 *P*<0.001**	**17.1 *P*<0.001**	**27.5 *P*<0.001**	**32.0 *P*<0.001**	**39.8 *P*<0.001**	**27.1 *P*<0.001**
B. pendula		3.64 *P*=0.74	**16.7 *P*<0.001**	**12.5 *P*<0.001**	**55.2 *P*<0.001**	**40.7 *P*<0.001**	**40.9 *P*<0.001**	**23.3 *P*<0.001**	**35.7 *P*<0.001**	**45.6 *P*<0.001**	**39.6 *P*<0.001**	**23.4 *P*<0.001**	**40.7 *P*<0.001**	**8.97 *P*<0.001**	**18.6 *P*<0.001**	**17.3 *P*<0.001**	**14.1 *P*=0.005**	3.15 *P*=0.29	**8.69 *P*=0.01**	**8.74 *P*=0.004**
C. sativa			**27.3 *P*<0.001**	**16.6 *P*<0.001**	**73.4 *P*<0.001**	**49.1 *P*<0.001**	**56.1 *P*<0.001**	**39.8 *P*<0.001**	**50.1 *P*<0.001**	**63.8 *P*<0.001**	**57.0 *P*<0.001**	**33.8 *P*<0.001**	**52.1 *P*<0.001**	**12.6 *P*<0.001**	**22.9 *P*<0.001**	**27.5 *P*<0.001**	**16.4 *P*=0.008**	3.74 *P*=0.44	**14.1 *P*=0.004**	**16.8 *P*<0.001**
Eucalyptus sp.				**21.5 *P*<0.001**	**16.4 *P*=0.02**	**13.6 *P*<0.001**	**16.0 *P*<0.001**	**20.6 *P*<0.001**	**26.9 *P*<0.001**	**44.6 *P*<0.001**	**40.2 *P*<0.001**	**23.3 *P*<0.001**	**41.6 *P*<0.001**	**7.15 *P*<0.001**	**12.8 *P*<0.001**	**7.30 *P*<0.001**	**23.9 *P*<0.001**	**15.7 *P*<0.001**	**22.7 *P*<0.001**	**7.24 *P*<0.001**
F. sylvatica					**44.0 *P*<0.001**	**30.7 *P*<0.001**	**22.8 *P*<0.001**	**49.2 *P*<0.001**	**54.4 *P*<0.001**	**79.2 *P*<0.001**	**76.3 *P*<0.001**	**34.6 *P*<0.001**	**49.5 *P*<0.001**	**9.10 *P*<0.001**	**6.88 *P*=0.02**	**19.3 *P*<0.001**	2.36 *P*=0.60	**6.28 *P*<0.001**	**1.65 *P*=0.51**	**15.3 *P*<0.001**
J. oxycedrus						**8.18 *P*<0.001**	**11.6 *P*=0.06**	**39.5 *P*<0.001**	**40.3 *P*<0.001**	**66.5 *P*<0.001**	**73.7 *P*<0.001**	**40.0 *P*<0.001**	**54.9 *P*<0.001**	**36.8 *P*<0.001**	**34.8 *P*<0.001**	**21.8 *P*<0.001**	**45.7 *P*<0.001**	**50.1 *P*<0.001**	**51.1 *P*<0.001**	**35.4 *P*<0.001**
J. thurifera							**11.5 *P*<0.001**	**44.6 *P*<0.001**	**49.7 *P*<0.001**	**80.0 *P*<0.001**	**86.2 *P*<0.001**	**43.6 *P*<0.001**	**61.1 *P*<0.001**	**23.4 *P*<0.001**	**20.7 *P*<0.001**	**23.1 *P*<0.001**	**28.0 *P*<0.001**	**31.3 *P*<0.001**	**40.4 *P*<0.001**	**32.3 *P*<0.001**
O. europea								**50.8 *P*<0.001**	**56.7 *P*<0.001**	**85.0 *P*<0.001**	**87.8 *P*<0.001**	**48.8 *P*<0.001**	**64.0 *P*<0.001**	**23.2 *P*<0.001**	**14.6 *P*=0.003**	**24.6 *P*<0.001**	**21.5 *P*<0.001**	**31.8 *P*<0.001**	**29.2 *P*<0.001**	**29.7 *P*<0.001**
P. halepensis									**13.0 *P*<0.001**	**11.2 *P*<0.001**	**12.9 *P*<0.001**	**16.1 *P*<0.001**	**31.5 *P*<0.001**	**37.6 *P*<0.001**	**54.3 *P*<0.001**	**20.1 *P*<0.001**	**52.3 *P*<0.001**	**36.4 *P*<0.001**	**43.1 *P*<0.001**	**22.1 *P*<0.001**

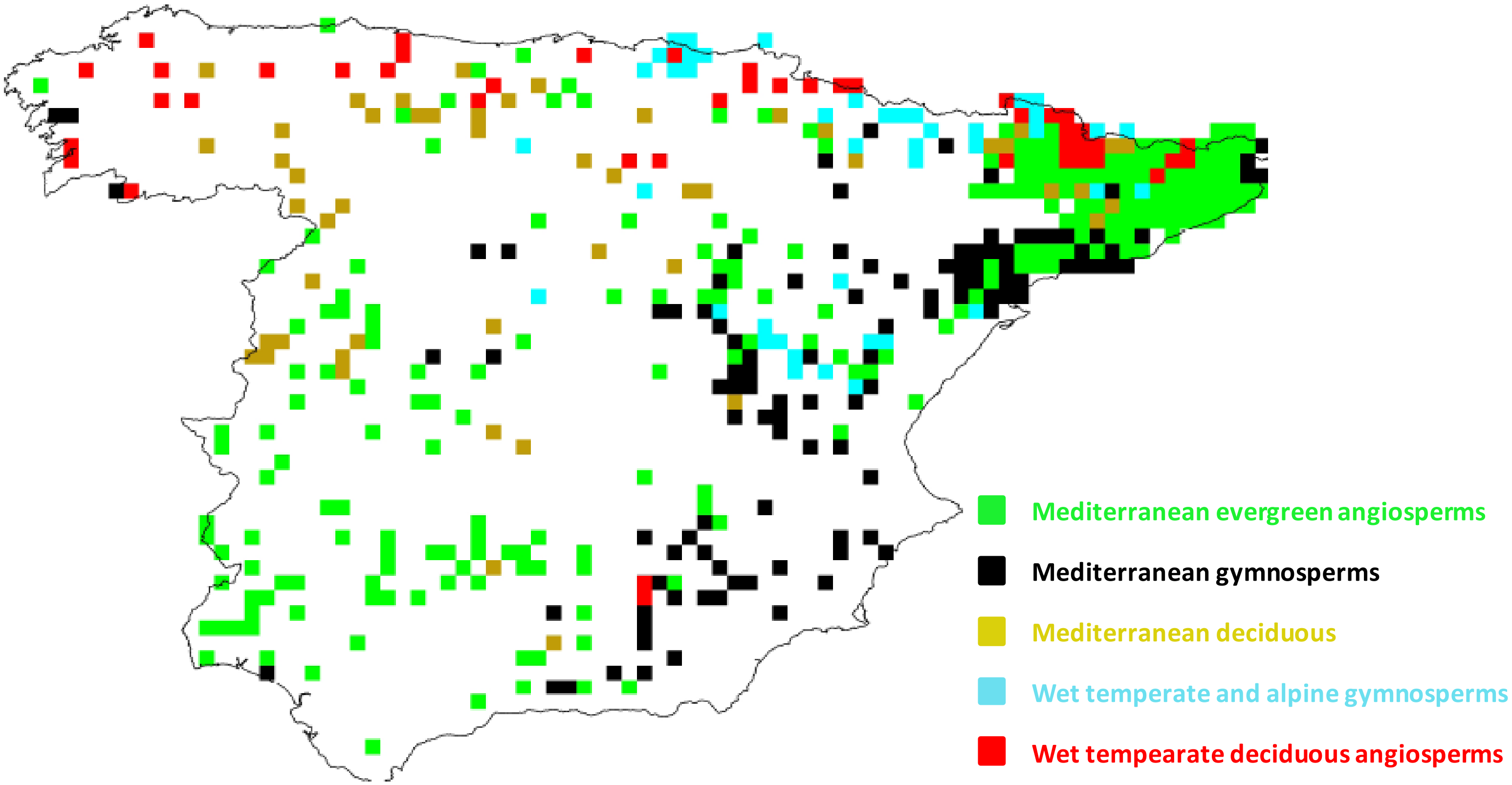
P. nigra										**6.64** **_P_<0.001**	**13.0** **_P_<0.001**	**3.48** **_P_<0.001**	**13.3** **_P_<0.001**	**45.8** **_P_<0.001**	**62.3** **_P_<0.001**	**12.0** **_P_<0.001**	**57.2** **_P_<0.001**	**44.5** **_P_<0.001**	**47.8** **_P_<0.001**	**19.7** **_P_<0.001**
P. pinaster											**4.40** **_P_=0.02**	**14.5** **_P_<0.001**	**24.9** **_P_<0.001**	**67.2** **_P_<0.001**	**90.3** **_P_<0.001**	**30.4** **_P_<0.001**	**83.2** **_P_<0.001**	**63.1** **_P_<0.001**	**67.4** **_P_<0.001**	**34.7** **_P_<0.001**
P.pinea												**20.0** **_P_<0.001**	**33.5** **_P_<0.001**	**58.0** **_P_<0.001**	**82.8** **_P_<0.001**	**30.8** **_P_<0.001**	**83.6** **_P_<0.001**	**58.1** **_P_<0.001**	**63.0** **_P_<0.001**	**28.7** **_P_<0.001**
P. sylvestris													**9.70** **_P_<0.001**	**33.2** **_P_<0.001**	**46.6** **_P_<0.001**	**6.52** **_P_<0.001**	**38.0** **_P_<0.001**	**29.1** **_P_<0.001**	**29.2** **_P_<0.001**	**12.1** **_P_<0.001**
P. uncinata														**52.6** **_P_<0.001**	**65.4** **_P_<0.001**	**19.5** **_P_<0.001**	**52.5** **_P_<0.001**	**46.3** **_P_<0.001**	**43.4** **_P_<0.001**	**27.2** **_P_<0.001**
Q. faginea															3.55 *P*=0.29	**14.1** **_P_<0.001**	**11.8** **_P_<0.001**	**4.65** **_P_<0.001**	**10.4** **_P_<0.001**	**7.41** **_P_<0.001**
Q. humilis																**21.4** **_P_<0.001**	8.15 *P*=0.10	**9.22** **_P_=0.001**	**11.2** **_P_=0.003**	**16.1** **_P_<0.001**
Q. ilex																	**23.2** **_P_<0.001**	**17.4** **_P_<0.001**	**17.8** **_P_<0.001**	**3.52** **_P_<0.001**
Q. petraea																		**5.83** **_P_=0.03**	5.13 *P*=0.19	**19.7** **_P_<0.001**
Q. pyrenaica																			**6.01** **_P_<0.001**	**9.91** **_P_<0.001**
Q. robur																				**11.8** **_P_<0.001**

Table 5. Test statistics for squared Mahalanobis distances between all pairwise comparisons of tree species from the discriminate functional analysis with log-transformed foliar concentrations of N, P, K, S, Ca and Mg and log-transformed N:P, N:K and P:K concentration ratios as variables using the 305 plots from the ICP data set dominated by the 13 most representative species of Mediterranean forest trees as cases. All pairwise distances are significant (*P*<0.05).

	Eucalyptus sp	*J. oxycedrus*	*J. thurifera*	*O. europea*	*P. halepensis*	*P. nigra*	*P. pinaster*	*P. pinea*	*Q. faginea*	*Q. ilex*	*Q. pyrenaica*	*Q. suber*
C.sativa	**24.6** **_P_<0.001**	**66.6** **_P_<0.001**	**43.7** **_P_<0.001**	**50.2** **_P_<0.001**	**40.4** **_P_<0.001**	**52.4** **_P_<0.001**	**68.1** **_P_<0.001**	**60.0** **_P_<0.001**	**11.2** **_P_=0.004**	**26.5** **_P_<0.001**	**3.38** **_P_<0.001**	**15.7** **_P_<0.001**
Eucalyptus sp.		**15.2** **_P_<0.001**	**12.6** **_P_<0.001**	**15.2** **_P_<0.001**	**20.8** **_P_<0.001**	**28.4** **_P_<0.001**	**46.8** **_P_<0.001**	**41.3** **_P_<0.001**	**7.14** **_P_<0.001**	**7.18** **_P_<0.001**	**14.5** **_P_<0.001**	**6.40** **_P_<0.001**
J. oxycedrus			**8.04** **_P_=0.04**	**11.9** **_P_=0.03**	**37.2** **_P_<0.001**	**38.4** **_P_<0.001**	**64.5** **_P_<0.001**	**70.1** **_P_<0.001**	**34.7** **_P_<0.001**	**19.2** **_P_<0.001**	**46.1** **_P_<0.001**	**31.5** **_P_<0.001**
J. thurifera				**10.6** **_P_<0.001**	**44.2** **_P_<0.001**	**50.2** **_P_<0.001**	**80.9** **_P_<0.001**	**84.9** **_P_<0.001**	**21.2** **_P_<0.001**	**21.4** **_P_<0.001**	**28.0** **_P_<0.001**	**29.0** **_P_<0.001**
O. europea					**51.0** **_P_<0.001**	**58.9** **_P_<0.001**	**88.0** **_P_<0.001**	**88.6** **_P_<0.001**	**20.9** **_P_<0.001**	**24.1** **_P_<0.001**	**28.3** **_P_<0.001**	**27.5** **_P_<0.001**
P. halepensis						**12.3** **_P_<0.001**	**11.1** **_P_<0.001**	**12.0** **_P_<0.001**	**39.5** **_P_<0.001**	**19.3** **_P_<0.001**	**38.2** **_P_<0.001**	**21.5** **_P_<0.001**
P. nigra							**6.54** **_P_<0.001**	**12.4** **_P_<0.001**	**49.9** **_P_<0.001**	**12.9** **_P_<0.001**	**48.3** **_P_<0.001**	**21.2** **_P_<0.001**
P. pinaster								**4.18** **_P_=0.01**	**72.6** **_P_<0.001**	**31.7** **_P_<0.001**	**68.7** **_P_<0.001**	**37.1** **_P_<0.001**

P. pinea		**62.2** ***P*<0.001**	**31.2** ***P*<0.001**	**62.1** ***P*<0.001**	**30.5** ***P*<0.001**
Q. faginea			**15.1** ***P*<0.001**	**4.11** ***P*<0.001**	**7.77** ***P*<0.001**
Q. ilex				**17.9** ***P*<0.001**	**3.46** ***P*<0.001**
Q. pyrenaica					**9.90** ***P*<0.001**

Figure 1


Mediterranean evergreen angiosperms
Mediterranean gymnosperms
Mediterranean deciduous
Wet temperate and alpine gymnosperms
Wet tempearate deciduous angiosperms

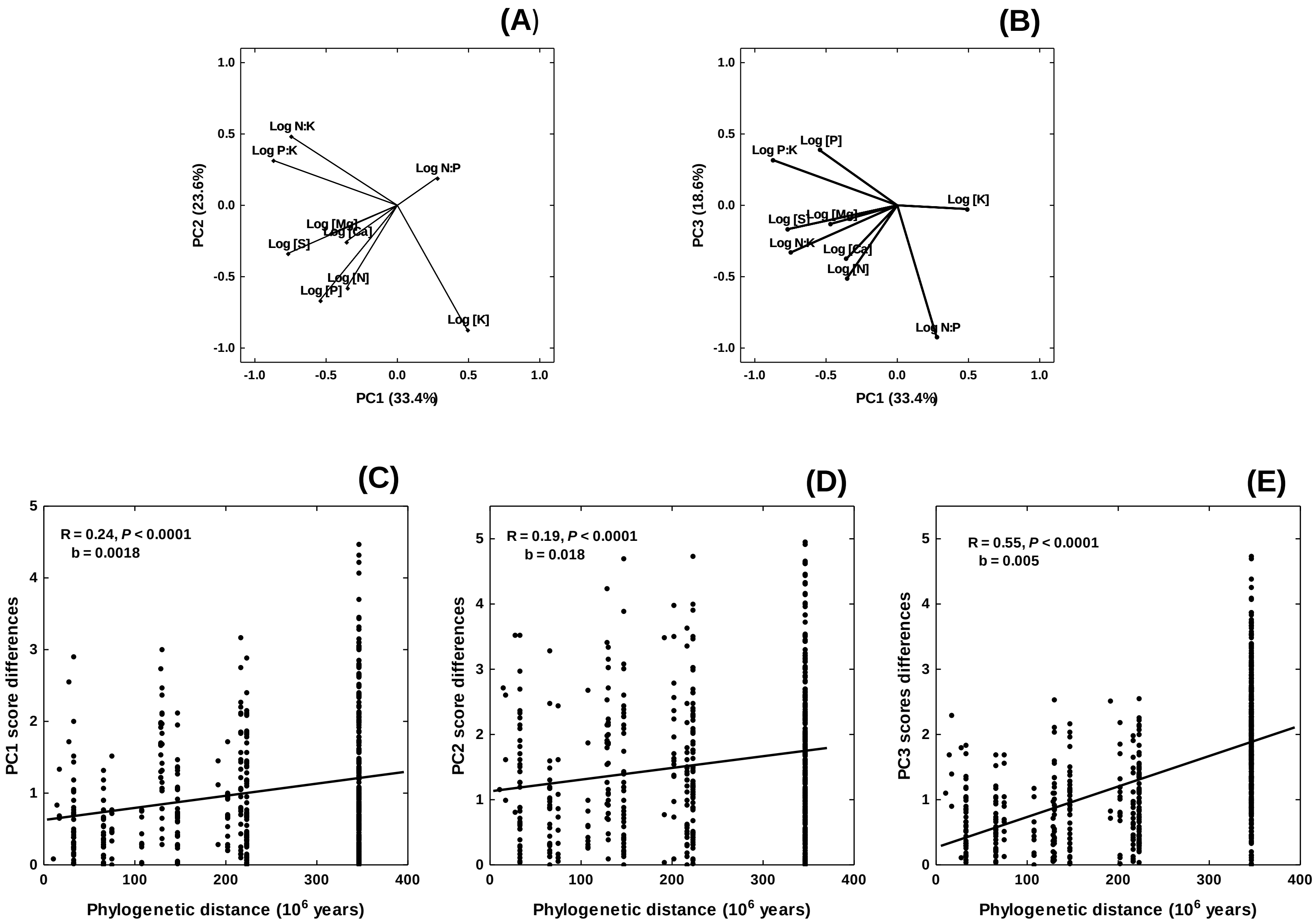

(A)
PC2 (23.6%)
PC1 (33.4%)
Log N:K
Log P:K
Log N:P
Log [Mg]
Log [Ca]
Log [S]
Log [N]
Log [P]
Log [K]
(B)
PC3 (18.6%)
PC1 (33.4%)
Log [P]
Log P:K
Log [K]
Log [S]
Log [Mg]
Log N:K
Log [Ca]
Log [N]
Log N:P
(C)
R = 0.24, P < 0.0001
b = 0.0018
PC1 score differences
Phylogenetic distance (10^6 years)
(D)
R = 0.19, P < 0.0001
b = 0.018
PC2 score differences
Phylogenetic distance (10^6 years)
(E)
R = 0.55, P < 0.0001
b = 0.005
PC3 scores differences
Phylogenetic distance (10^6 years)

Figure 2

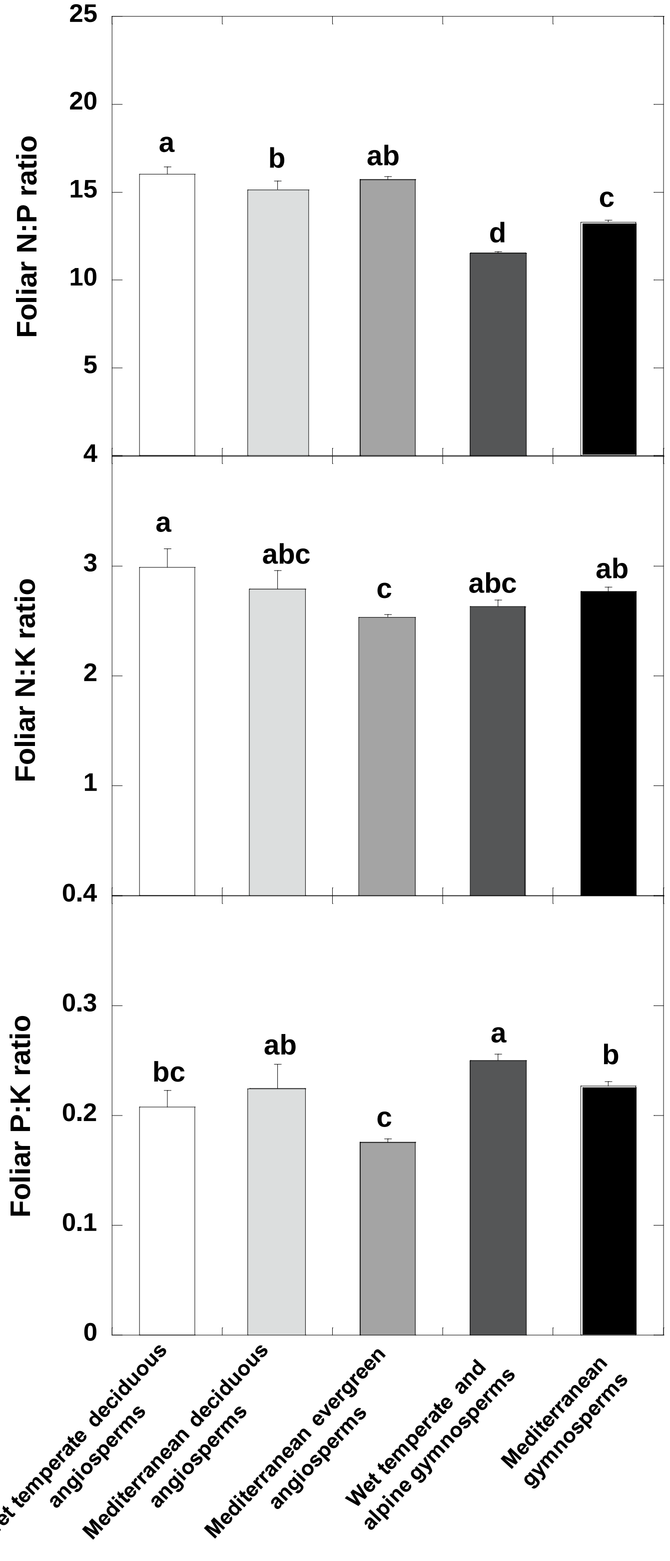

Foliar N:P ratio
25
20
15
10
5
4
a
b
ab
d
c
Foliar N:K ratio
3
2
1
0.4
a
abc
c
abc
ab
Foliar P:K ratio
0.4
0.3
0.2
0.1
0
bc
ab
c
a
b
Wet temperate deciduous angiosperms
Mediterranean deciduous angiosperms
Mediterranean evergreen angiosperms
Wet temperate and alpine gymnosperms
Mediterranean gymnosperms

Figure 3

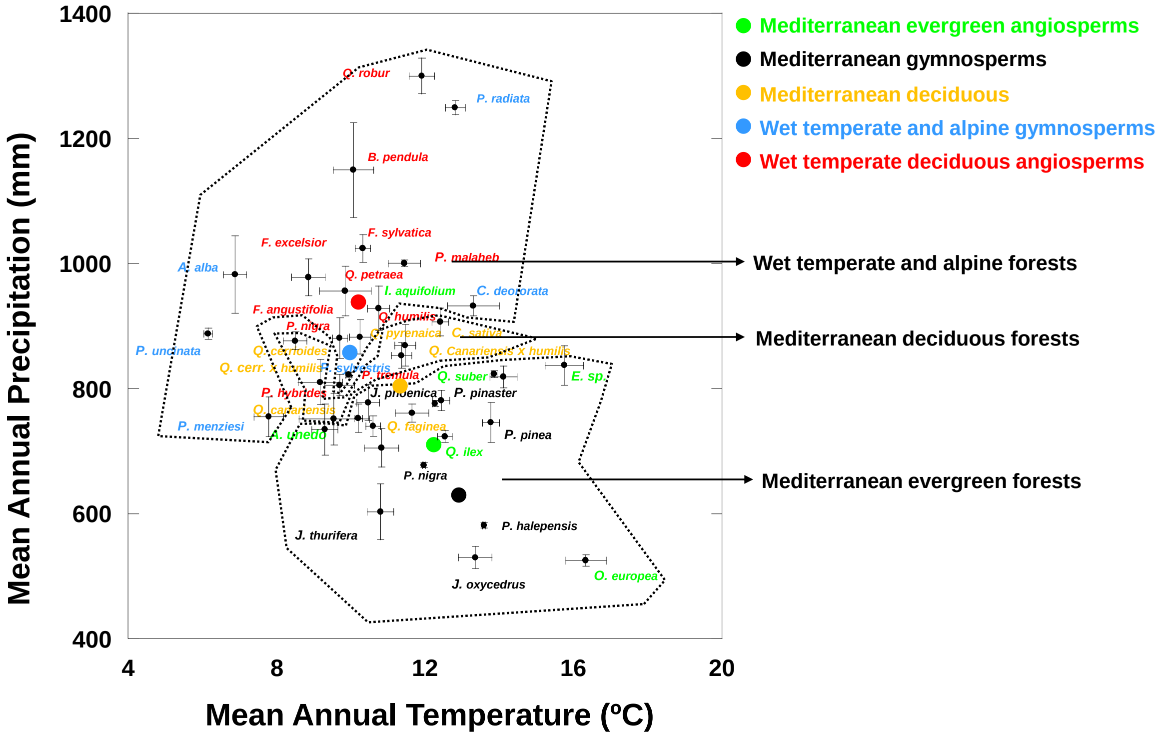

Mediterranean evergreen angiosperms
Mediterranean gymnosperms
Mediterranean deciduous
Wet temperate and alpine gymnosperms
Wet temperate deciduous angiosperms
Wet temperate and alpine forests
Mediterranean deciduous forests
Mediterranean evergreen forests
Mean Annual Precipitation (mm)
1400
1200
1000
800
600
400
Mean Annual Temperature (ºC)
4
8
12
16
20
Q. robur
P. radiata
B. pendula
F. sylvatica
F. excelsior
P. malaheb
A. alba
Q. petraea
I. aquifolium
C. deodorata
F. angustifolia
Q. humilis
P. nigra
Q. pyrenaica
C. sativa
P. uncinata
Q. cerrioides
Q. Canariensis x humilis
Q. cerr. x humilis
P. sylvestris
P. tremula
Q. suber
E. sp.
P. hybrides
J. phoenica
P. pinaster
Q. canariensis
P. menziesi
A. unedo
Q. faginea
P. pinea
Q. ilex
P. nigra
P. halepensis
J. thurifera
J. oxycedrus
O. europea

Figure 4

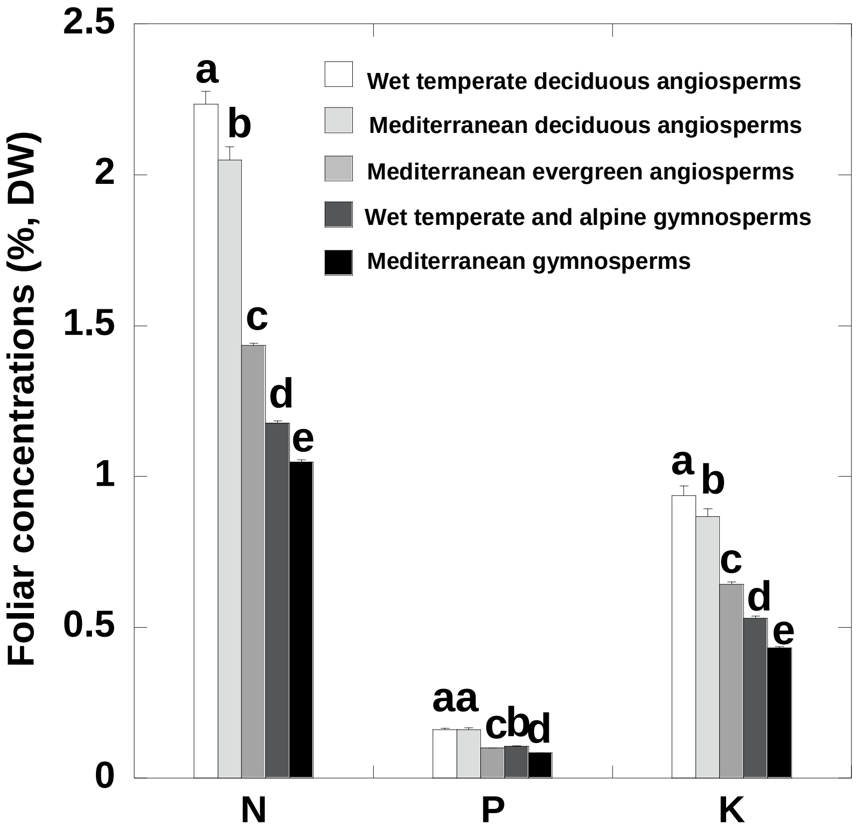

2.5
2
1.5
1
0.5
0
Foliar concentrations (%, DW)
N
P
K
a
b
c
d
e
aa
cbd
a
b
c
d
e
Wet temperate deciduous angiosperms
Mediterranean deciduous angiosperms
Mediterranean evergreen angiosperms
Wet temperate and alpine gymnosperms
Mediterranean gymnosperms

Figure 5

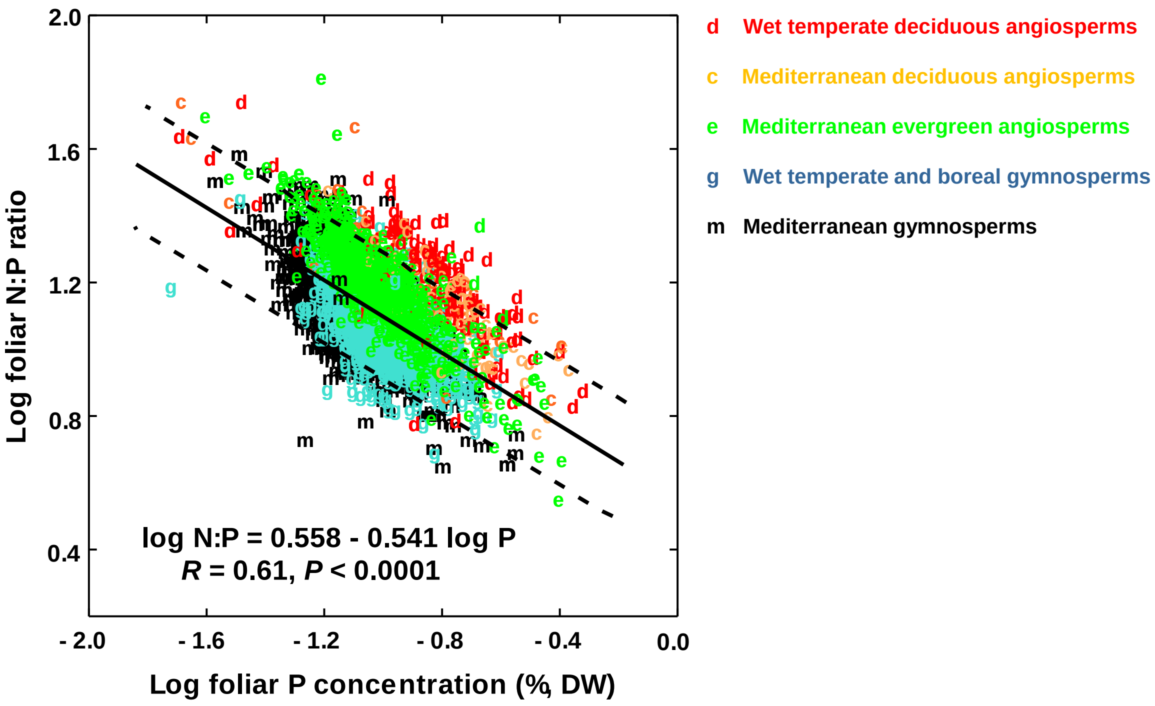
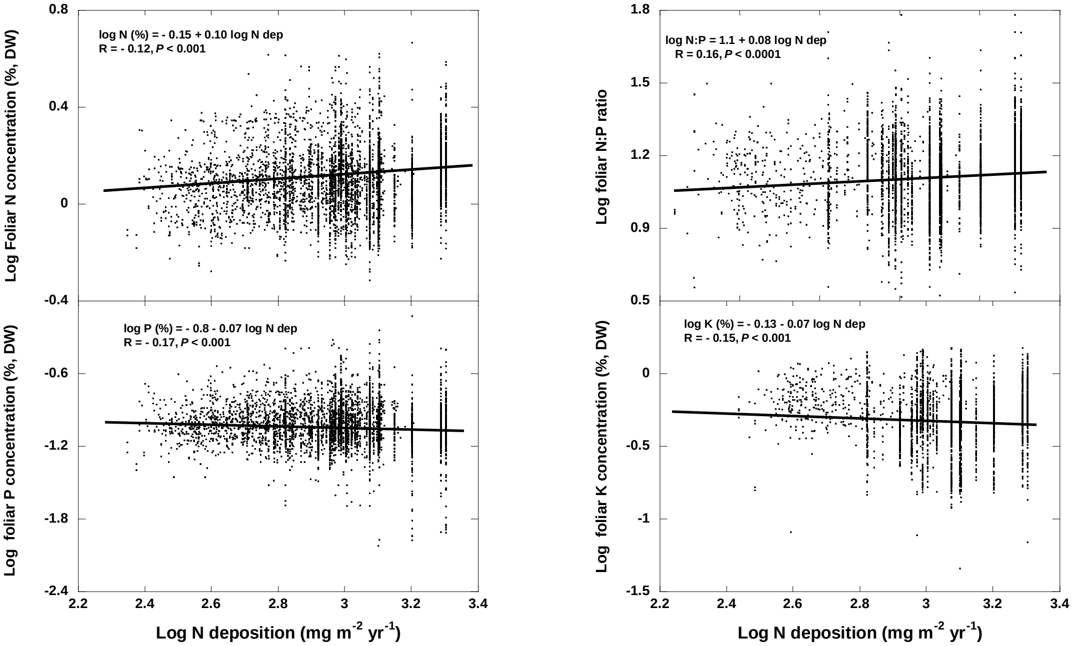

Log foliar N:P ratio
2.0
1.6
1.2
0.8
0.4
- 2.0
- 1.6
- 1.2
- 0.8
- 0.4
0.0
Log foliar P concentration (%, DW)
log N:P = 0.558 - 0.541 log P
R = 0.61, P < 0.0001
d Wet temperate deciduous angiosperms
c Mediterranean deciduous angiosperms
e Mediterranean evergreen angiosperms
g Wet temperate and boreal gymnosperms
m Mediterranean gymnosperms

Figure 6

Figure 7



log N (%) = - 0.15 + 0.10 log N dep
R = - 0.12, P < 0.001
Log Foliar N concentration (%, DW)
0.8
0.4
0
-0.4
log N:P = 1.1 + 0.08 log N dep
R = 0.16, P < 0.0001
Log foliar N:P ratio
1.8
1.5
1.2
0.9
0.5
log P (%) = - 0.8 - 0.07 log N dep
R = - 0.17, P < 0.001
Log foliar P concentration (%, DW)
-0.6
-1.2
-1.8
-2.4
log K (%) = - 0.13 - 0.07 log N dep
R = - 0.15, P < 0.001
Log foliar K concentration (%, DW)
0
-0.5
-1
-1.5
2.2
2.4
2.6
2.8
3
3.2
3.4
Log N deposition (mg m-2 yr-1)
Log N deposition (mg m-2 yr-1)

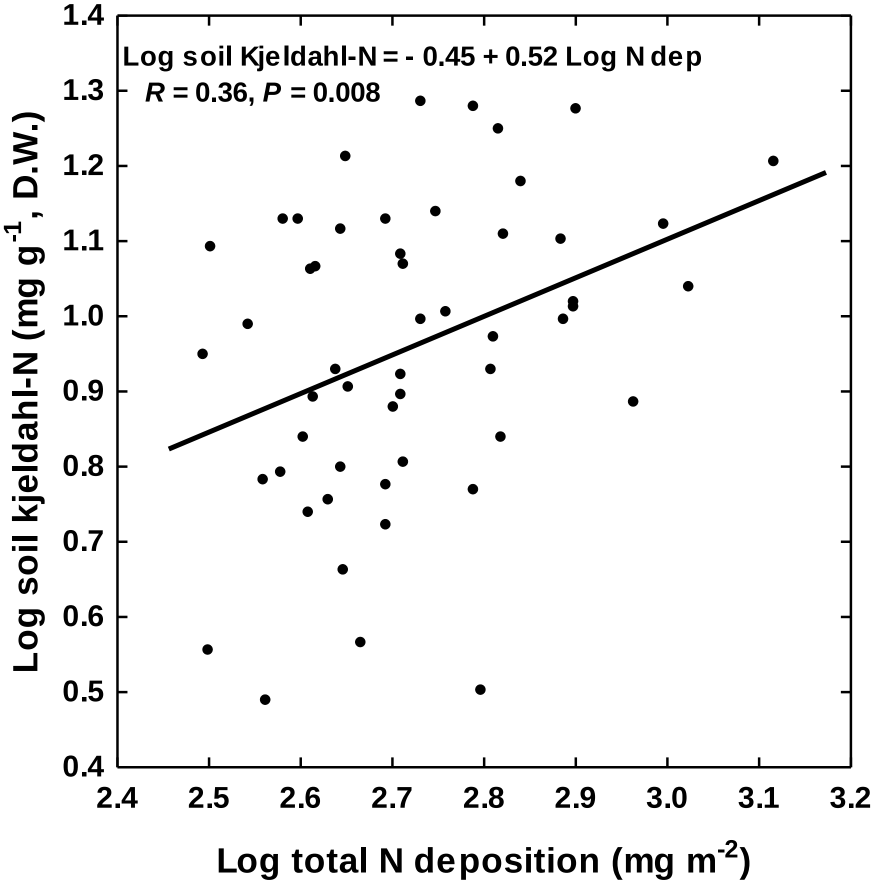
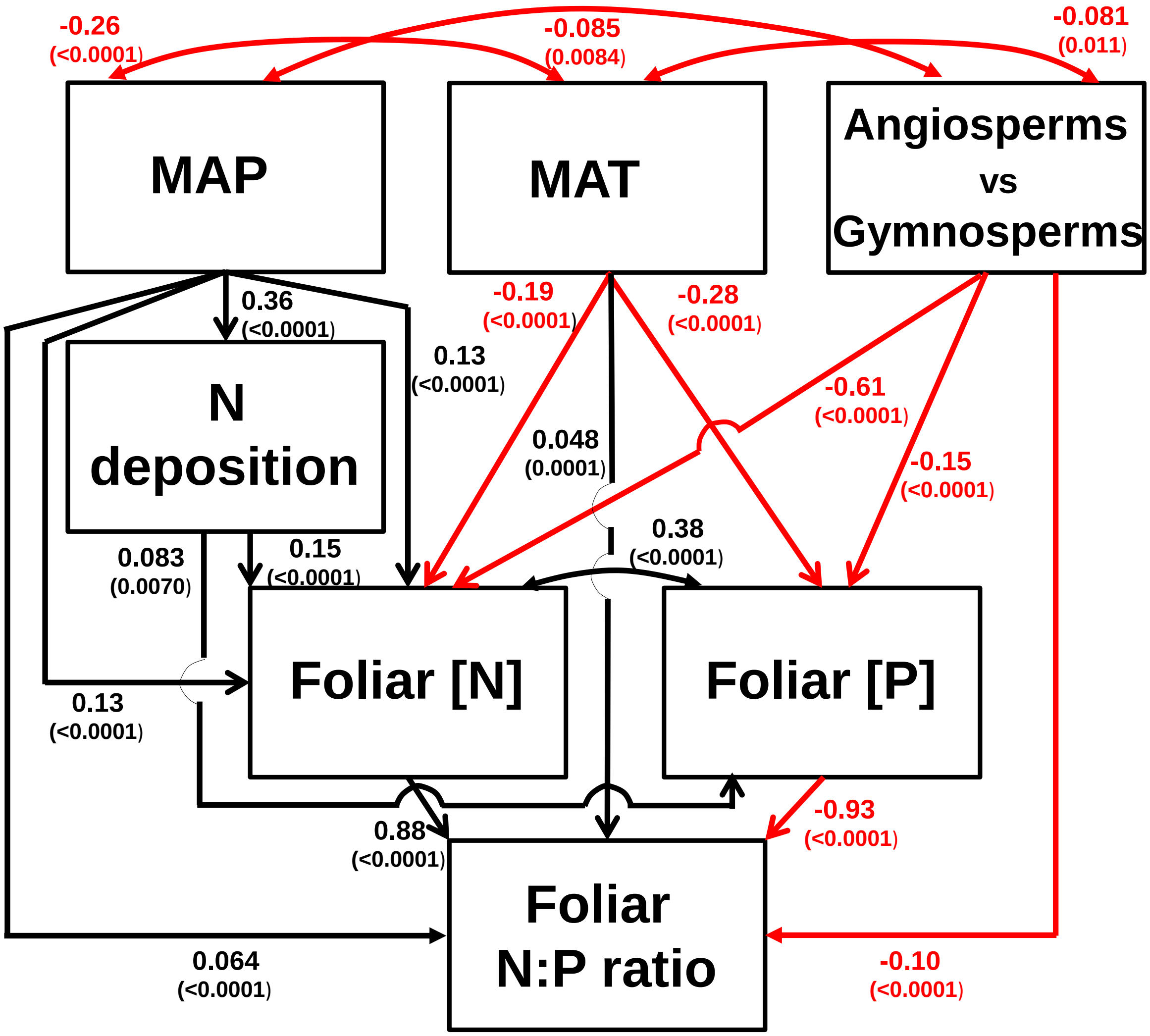

Log soil Kjeldahl-N = - 0.45 + 0.52 Log N dep
R = 0.36, P = 0.008
Log soil kjeldahl-N (mg g^{-1}, D.W.)
Log total N deposition (mg m^{-2})
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2

Figure 8



R^2 for endogenous variables

N deposition	Foliar [P]	Foliar [N]	foliar N:P ratio
0.13	0.10	0.47	0.88

Figure 9

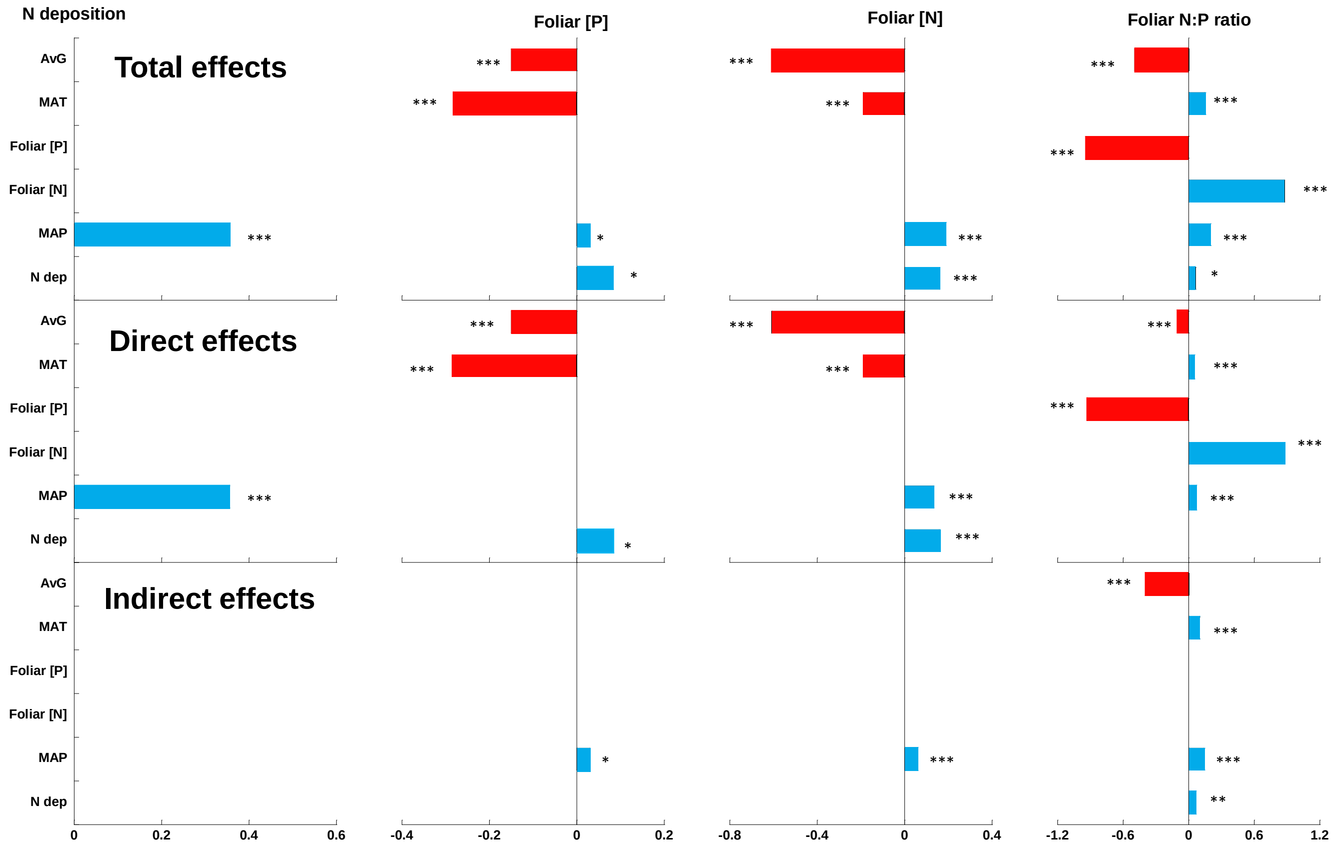

N deposition
Foliar [P]
Foliar [N]
Foliar N:P ratio
Total effects
Direct effects
Indirect effects
AvG
MAT
Foliar [P]
Foliar [N]
MAP
N dep
***
**
*
0
0.2
0.4
0.6
-0.4
-0.2
-0.8
-1.2
-0.6
1.2

Figure 10

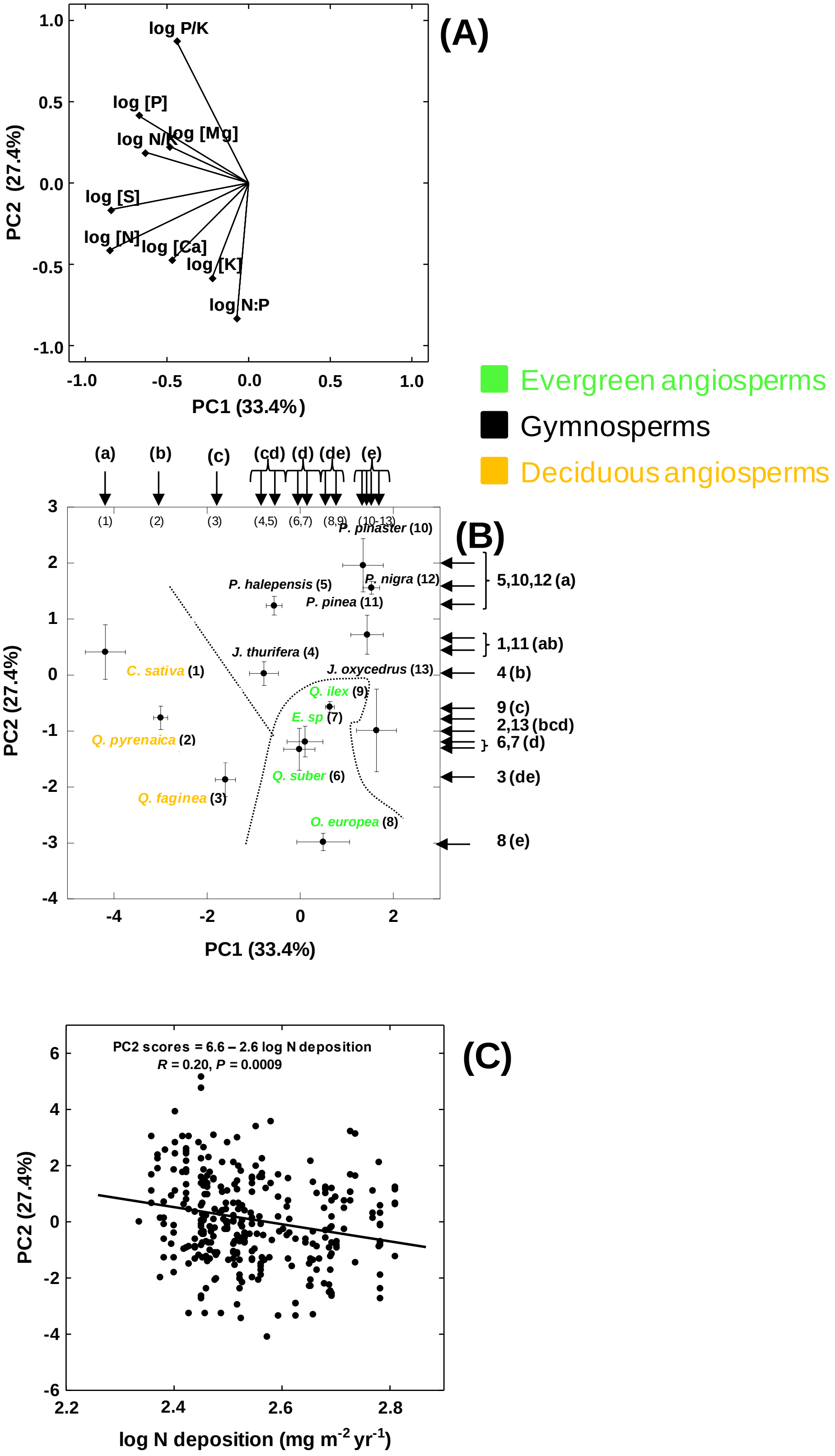

(A)
log P/K
log [P]
log N/K
log [Mg]
log [S]
log [N]
log [Ca]
log [K]
log N:P
PC2 (27.4%)
PC1 (33.4%)
Evergreen angiosperms
Gymnosperms
Deciduous angiosperms
(B)
(a) (b) (c) (cd) (d) (de) (e)
(1) (2) (3) (4,5) (6,7) (8,9) (10-13)
P. pinaster (10)
P. nigra (12)
P. halepensis (5)
P. pinea (11)
J. thurifera (4)
J. oxycedrus (13)
C. sativa (1)
Q. ilex (9)
E. sp (7)
Q. pyrenaica (2)
Q. suber (6)
Q. faginea (3)
O. europea (8)
5,10,12 (a)
1,11 (ab)
4 (b)
9 (c)
2,13 (bcd)
6,7 (d)
3 (de)
8 (e)
PC2 (27.4%)
PC1 (33.4%)
(C)
PC2 scores = 6.6 – 2.6 log N deposition
R = 0.20, P = 0.0009
PC2 (27.4%)
log N deposition (mg m-2 yr-1)

Figure 11

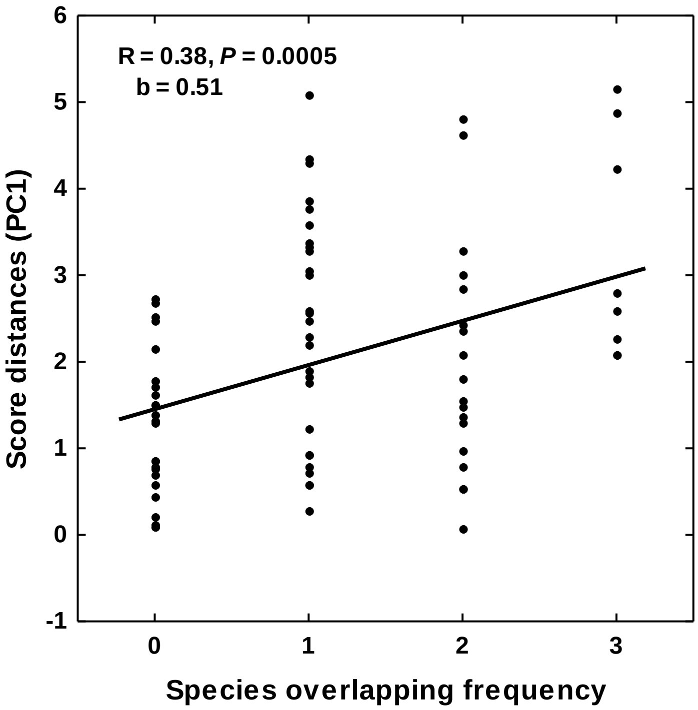

R = 0.38, P = 0.0005
b = 0.51
Score distances (PC1)
6
5
4
3
2
1
0
-1
0
1
2
3
Species overlapping frequency

Figure 12