Foliar and soil concentrations and stoichiometry of nitrogen and phosphorous across European *Pinus sylvestris* forests: relationships with climate, N deposition and tree growth

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**Summary**

1. This study investigated the factors underlying the variability of needle and soil elemental composition and stoichiometry and their relationships with growth in *Pinus sylvestris* forests throughout the species’ distribution in Europe by analysing data from 2245 forest stands.

2. Needle N concentrations and N:P ratios were positively correlated with total atmospheric N deposition, whereas needle P concentrations were negatively correlated. These relationships were especially pronounced at sites where high levels of N deposition coincided with both higher mean annual temperature and higher mean annual precipitation. Trends towards foliar P deficiency were thus more marked when high N deposition coincided with climatic conditions favourable to plant production.

3. Atmospheric N deposition was positively correlated with soil solution NO\(_3^-\), SO\(_4^{2-}\), K\(^+\), P and Ca\(^{2+}\) concentrations, the soil solution NO\(_3^-\):P ratio, total soil N and the total soil N:Olsen P ratio and was negatively correlated with soil Olsen P concentration.

4. Despite these nutrient imbalances, during the period studied (1990–2006), N deposition was positively related with *Pinus sylvestris* absolute basal diameter (BD) growth, although only accounting for the 10% of the total variance. However, neither N deposition nor needle N concentration was related with relative annual BD growth. In contrast, needle P concentration was positively related with both absolute and relative annual BD growth.

5. These results thus indicate a tendency of European *P. sylvestris* forests to store N in trees and soil in response to N deposition and unveil a trend towards increased nutrient losses in run-off as a consequence of higher soil solution N concentrations. Overall, the data show increasing ecosystem nutrient imbalances with increasingly limiting roles of P and other nutrients such as K in European *P. sylvestris* forests, especially in the centre of their distribution where higher levels of N deposition are observed. Thus, although the data show that N deposition has had an overall positive effect on *P. sylvestris* growth, the effect of continuous N deposition, associated with decreasing P and K and increasing N:P
in leaves and in soil, may in the future become detrimental for the growth and competitive ability of *P. sylvestris* trees.

**Key-words:** C:N ratio, C:P ratio, ecological stoichiometry, foliar nutrient content, global change, K, N deposition, N:P ratio, soil eutrophication, soil solution

**Introduction**

The foliar N:P ratio is associated with several ecological traits and processes, such as the growth strategies of species (Willby, Pulford & Flowers 2001), species diversity (Seastedt & Vaccaro 2001; Güsewell *et al.* 2005) and ecosystem fluxes, functions and compartmental structure (Elser *et al.* 1996, 2010; Sterner & Elser 2002; Hughes & Denslow 2005). The foliar N:P ratio is also an essential indicator of plant nutritional status and productive capacity (Güsewell 2004; McGroddy, Daufresne & Hedin 2004; Sardans, Rivas-Ubach & Peñuelas 2012a). The spatial and temporal patterns of N and P concentrations and of N:P ratios in terrestrial plants have thus been studied extensively on regional and global scales (Vitousek, Turner & Kitayama 1995; Oleksyn *et al.* 2003; Reich & Oleksyn 2004; Richardson, Allen & Doherty 2008; Sardans, Rivas-Ubach & Peñuelas 2011, 2012a; Sardans & Peñuelas 2013).

These studies have typically been carried out as analyses of data from a large number of species. In contrast, analyses of stoichiometric differences within single species are much less common. Khang *et al.* (2011) studied the differences in N and P concentrations and the N:P ratio in the needles of *Picea abies* in several European countries and observed higher N and P concentrations in the central part of the tree’s distribution, suggesting that higher needle N and P concentrations are associated with the optimum climatic conditions for biomass production. In line with this, both *P. abies* and *P. sylvestris* have higher growth and survival rates in the centres of their distributions (Sykes & Prentice 1995). Anthropogenic eutrophication of ecosystems as a result of atmospheric N deposition and climate change can alter plant N and P concentrations and the N:P ratios of soils, water and organismic tissues (Elser *et al.* 2010; Peñuelas *et al.* 2012, 2013; Sardans, Rivas-Ubach & Peñuelas 2012a). A growing number of studies have reported an increase in the N:P ratios of aquatic and terrestrial plants in response to increasing N loadings (Peñuelas *et al.* 2012, 2013; Sardans, Rivas-Ubach & Peñuelas 2012a). N deposition can also affect soil properties, including N concentrations (Rustad *et al.* 1993; Verstraeten *et al.* 2012), and cause nutrient imbalances (Fujita *et al.* 2010), which have not yet been globally assessed (Peñuelas *et al.* 2012, 2013; Sardans, Rivas-Ubach & Peñuelas 2012b). Atmospheric N deposition accelerates the N cycle, increasing N availability, while other essential nutrients (P, K, Ca, Mg) may become limiting factors (Sutton *et al.* 2011). Changes in plant N:K ratios caused by N deposition have been observed in European *Calluna vulgaris* heathlands (Britton *et al.* 2008; Rowe *et al.* 2008). Sulphur (S) deposition is frequently associated with N deposition (Van Dijk *et al.* 2012; Eherne & Posch 2013), changing soil and plant elemental composition and stoichiometry (Robroek *et al.* 2009; Van Dijk *et al.* 2012). This further affects the demographic, phenotypically plastic and adaptive responses of plants (Carnicer *et al.* 2015), so S and N deposition are usually studied together to analyse the impacts of human-driven global change on the plant–soil system.

Stoichiometric flexibility has been defined as the ability of organisms to adjust their elemental ratios while maintaining constant functions (Sistla & Schimel 2012). This flexibility can act at different scales, from individual organisms at the physiological level (e.g. reallocation and changes in uptake strategies) and ontological level to communities and ecosystems (e.g. changes in species composition). Most plants have some degree of stoichiometric flexibility (Güsewell 2004), but most terrestrial plant species are less stoichiometrically flexible than plankton are, and some even exhibit considerable stoichiometric homeostasis. Little is known, however, about the capacity of terrestrial plant species to have flexible stoichiometries along their natural ranges of distribution in response to environmental changes is necessary to obtain knowledge of stoichiometric flexibility and its ecological significance. These studies should allow us to understand the role of stoichiometric flexibility, as opposed to stoichiometric homeostasis, and its relationship with ecological characteristics of species, such as growth strategy, capacity to adapt to environmental changes and nutrient-use efficiency. Moreover, as recent reviews suggest, the role played by stoichiometric flexibility in linking micro- to macro-scale biogeochemical cycles in a changing world requires investigation (Sistla & Schimel 2012). In this sense, anthropogenic processes, such as those that increase N deposition, can also affect plant stoichiometry both directly and by interacting with other environmental variables such as climatic variables. Thus, we hypothesized that atmospheric N deposition can change needle elemental composition in *P. sylvestris* forests across Europe and that this, at least in part, is related to the effects of N deposition on soil chemistry.

*Pinus sylvestris* is one of the most important forest species in Eurasia, with a broad distribution extending from Spain to Siberia (Cheddadi *et al.* 2006). This distribution across Europe offers a unique opportunity to study the relationships of soil and plant nutrient concentrations and stoichiometry with climate, atmospheric N deposition, and plant growth. A previous study, using a data set of 195
stands of *P. sylvestris* across Europe, reported a trend towards increased needle N concentrations with latitude (Oleksyn *et al.* 2003). Some of these data, however, were obtained in the 1960s, before the dramatic increase in N deposition due to human activities (Prietzel & Stetter 2010). The considerable climatic gradients (mean annual precipitation (MAP), 335–1768 mm year$^{-1}$; mean annual temperature (MAT), -2.1 to 16.1 °C) and the variation in N deposition (0.55–27.0 kg N ha$^{-1}$) across the distribution of *P. sylvestris* make this species an adequate model to test the links between these variables and their interactions, growth and the soil–plant stoichiometric response. Furthermore, we aimed to investigate whether the effects of climatic variables and N deposition on *P. sylvestris* N:P ratio can be due, at least partially, to the indirect effects of changing soil conditions. We hypothesized that N deposition could have contributed to imbalanced elemental composition of soil and foliage in *P. sylvestris* forests across Europe, and further that this could probably be linked to changes in growth patterns resulting from the balance between the positive fertilization effect and the negative nutrient imbalance effect.

In this study, we investigated the needle–soil elemental composition and stoichiometric flexibility of *P. sylvestris* forests and their imbalances in response to climatic gradients and atmospheric N deposition. We also probed for additional relationships of those variables with *P. sylvestris* growth by analysing a compiled data set of 2245 stands of *P. sylvestris* throughout Europe, obtained mainly from 1990 to 2006. The specific objectives of this study were to discern (i) the patterns of needle N, P, K and S concentrations and N:P ratio throughout the European distribution of *P. sylvestris*, (ii) the allometric relationships between needle N and P concentrations that could possibly affect N:P ratios, (iii) the impacts of climate and N deposition on needle and soil N, P, K, Ca, Mg and S concentrations and N:P ratio, (iv) the effects of temporal trends of atmospheric N deposition on needle N, P, K, Ca, Mg and S concentrations and (v) the effects of N deposition and needle nutrient concentrations on growth of *P. sylvestris*.

**Material and methods**

**EUROPEAN DATA FOR NEEDLE AND SOIL NUTRIENT CONCENTRATIONS**

Data for needle N and P concentrations of Scots pine (*P. sylvestris*) were obtained from the Web of science, http://apps.webofknowledge, the ICP Forests monitoring programme (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests, operated under the UNECE Convention on Long-range Transboundary Air Pollution, http://icp-forests.net; Fisher *et al.* 2012), the TRY data base (Kattge *et al.* 2011), the Ecological Forest Inventory of Catalonia (Gracia *et al.* 2004) and the Third Spanish National Forest Inventory (Villanueva 2005). The data only included sites where *P. sylvestris* was a pure stand or the dominant tree species. The data were harmonized by selecting data from studies that determined N concentration by Kjeldahl and/or dry combustion, used a Carlo Erba elemental analyser, and determined P, K, Ca, Mg and S concentrations by atomic spectrometric methods. Needle N:P ratios were based on mass. We obtained needle data for 2245 forest plots of *P. sylvestris* spread across its entire range in Europe with samples collected between 1990 and 2006 (Fig. 1). Additionally, data for needle N, P and S concentrations for the period 1994–2007 in the ICP Forests data base were available for 90 *P. sylvestris* plots distributed across twelve European countries. These data were used to study the temporal shifts in needle nutrient concentrations. Data for needle N, P, K, Ca and Mg concentrations from the ICP Forests data base were also available for 119 plots from 16 countries, and annual absolute and relative basal diameter (BD) growth were available for 189 plots from 18

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countries in the period 1995–2005. To compare growth and needle elemental composition with climate and N loadings, we also collected the climate and N loadings (throughfall N deposition and MAP from ICP) for 82 of the 189 plots from which growth data were available for the same plots and periods. To compare needle elemental composition with climate and N loadings (throughfall N deposition and MAP from ICP), we also collected throughfall and MAP data from 55 of the 119 plots from which needle elemental composition data were available for the same plots and periods. Absolute DB growth was calculated as the average stand diameter annual growth, whereas diameter was measured at breast height and the relative DB growth is the absolute stand diameter growth relative to the initial stand BD.

We also used the soil solution chemistry (NO$_3$–, K$^+$, Ca$^{2+}$ and SO$_4^{2–}$ of organic horizons) of 67 P. sylvestris plots (Fig. S1, Supporting information) monitored under the ICP Forests Intensive Forest Monitoring (level II) from 1995 to 2005; P concentration in soil solution of the organic horizons was available for 54 of these 67 plots. In these 67 plots, needle S, N and P and N:P ratio data were available for the same plots and periods and we were able to analyse the relationships between soil NO$_3$– and needle elemental composition. N throughfall and MAP data were available for the same plots and periods in 4167 plots (from ICP Forests Intensive Forest Monitoring). Data for total soil N, Ca, K and Olsen P (a proxy for soil P available to plants) concentrations were available for 57 plots monitored under the ICP Forests Intensive Forest Monitoring (level II) from 1995 to 2005. Data on soil variables and N throughfall and MAP from ICP were available in 36 plots for the same plots and periods. We used these data to analyse the relationships between soil chemistry and climate (WorldClim data base and ICP Forests), and N deposition [from European Monitoring and Evaluation Programme (EMEP) and ICP Forests]. These data were used to study the relationships between absolute and relative annual BD growth, climate (from WorldClim data base and using MAP from the same plots), atmospheric N deposition (from EMEP and from ICP Forests) and needle elemental composition. We had soil taxonomic information for 32 plots: most soils were podzols (11) and arenosols (19), and only one plot had umbrisols and another cambisols. We have analysed the differences in needle elemental concentration and ratios and in some available soil variables between podzols and arenosols. All georeferenced data were processed using MIRAMON 6.0 (Pons 2009). All commented statistical analyses with the corresponding data sources, and Figures and Tables showing results are presented in Table 1.

**CLIMATIC AND N DEPOSITION DATA**

For each stand, we extracted climatic data derived from the WorldClim data base (Hijmans et al. 2005). This data base provides global maps of interpolated variables of mean temperature and precipitation calculated from an extensive climatic time series (from 1950 to 2000), with a spatial resolution of 30 arc seconds (≥1 km at the equator). We used MAT and MAP as climatic predictor variables.

Data for atmospheric N deposition were obtained from the 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 eutrophying compounds (SO$_2$–, N), ground-level ozone (O$_3$) and particulate matter (PM$_{10}$, PM$_{2.5}$). This study used data for total atmospheric N and S deposition estimated annually for the period 1990–2006 with EMEP model v3r8.1 over Europe using a grid size of 50 km × 50 km (EMEP, 2011). In Fig. S2 are shown the N-NO$_3$– and NH$_4$+ in bulk deposition (kg ha$^{-1}$) of the year 2011 in Europe.

**DATA ANALYSES**

We used simple regression analysis to study the scaling relationships between needle N and P concentrations. For this analysis, we used the unbiased Theil–Sen’s slope estimator (Theil 1950; Sen 1968) and the mbhm package (median-based linear models, Komsta 2012) from the R software (R Core team 2013) to avoid outlier influence in the bivariate fits.

To check the relationships between all studied variables (climatic, atmospheric N deposition, needle and soil variables), we used multiple correlations performed with STATISTICA 6.0 (StatSoft, Inc. Tule, OK, USA). In the multiple correlations, we applied the Bonferroni correction to adjust confidence intervals and P values. We used the r package mgcv (Wood 2000) for generalized additive models to smooth the trends of [N], log [P], log N:P and log N deposition with latitude by means of thin-plate spline regressions. We also conducted principal component analyses to thereafter correlate PC scores, as proxies of overall needle elemental composition, with growth.

We applied general linear models (GLMs) to analyse the relationships of climatic variables (MAP and MAT) with atmospheric N deposition and their interactions on needle N, P, K and S concentrations and N:P ratios and on soil and soil solution nutrient concentrations and stoichiometries. Needle N concentration was normally distributed, whereas P concentration and the N:P ratio were not. To normalize their distributions, these variables were log-transformed in the analyses. In the models used to analyse the relationships between MAP, MAT and N deposition with the needle and soil variables and growth, as well as the relationships between needle variables and growth, the 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 Carncier et al. 2011). To assess the level of significance at each distance class in the Moran’s I correlograms, we computed 1000 permutations using the resamp argument in the correlog function (ncf package, R software). When significant autocorrelation in the residuals was detected in ordinary least squares (OLS) models, we applied spatial statistical modelling methods (spatial simultaneous autoregressive (SAR) models) to remove, or at least reduce, the spatial autocorrelation in the residuals of the models. In these models, we used MAP, MAT and total atmospheric N deposition as continuous independent variables. We used a factorial design, but in the final models we only maintained the interactions among the significant independent variables.

To detect the possible associations between climate traits and needle elemental composition via direct relationships but also by indirect relation through N deposition, we used structural equation modelling (SEM). We fitted the different models using the sem package (Fox et al. 2013) and achieved the minimum adequate model using the AIC. Standard errors and the level of significance (P-value) of the total, direct and indirect relationships were calculated using the bootstrap (with 1200 repetitions) technique (Davison, Hinkley & Schechtman 1986; Mitchell-Olds 1986).

We used 1027 records from ICP Forests data of NO$_3$–, NH$_4$+, SO$_4^{2–}$ and total N in throughfall of P. sylvestris forests over the period of 1995–2011 in 98 different sites in 18 European countries. We used this data to analyse the relationships between these throughfall data from ICP Forests and the corresponding data (in the same sites and years) of bulk deposition from the EMEP model. In this way, we analysed whether or not EMEP data accurately determined, without under- or overestimation, the loadings of N and S in forest soils.

Finally, we assessed the temporal shifts (from 1994 to 2007) of needle N, P and S concentrations for 90 plots of the ICP Forests data distributed over 12 European countries; using STATISTICA 6.0, we performed a one-way analysis of variance with different years as independent variables and needle N, P and S concentrations as dependent variables.

Both needle N and P concentrations have significant variability within the latitudinal gradient, but both were higher in the central latitudes within the area of distribution. The thin-plate spline regressions showed maximum needle N concentrations of $2.34 \pm 0.1$ (%, DW) at $48^\circ$N (Figs. 1a and 2a), whereas needle P concentrations increased beginning from south Europe to mid-latitudes and then had relatively constant values with increasing latitude (Fig 2b).
Needle N:P ratios, however, tended to decrease from low to high latitudes (Figs. 1b and 2c). MAP, MAT and total atmospheric N deposition ranged from 733 to 1768 mm year$^{-1}$, from $-2.1$ to 16.1 °C and from 0.55 to 27 kg ha$^{-1}$ year$^{-1}$, respectively (Hijmans et al. 2005; Simpson et al. 2012). On a log-log scale, needle N concentrations scaled at 0.423 relative to needle P concentrations (Fig. 3a), indicating that P concentrations increased proportionally more than needle N concentrations when both concentrations increased (Fig. 3b).

**RELATIONSHIPS BETWEEN NEEDLE N, P AND S CONCENTRATIONS AND N:P RATIOS WITH ATMOSPHERIC N DEPOSITION AND CLIMATE**

Within the European distribution of *P. sylvestris* forests, maximum atmospheric N deposition occurred near 52 °N (Fig. S3), near the observed latitude of maximum needle N concentration of 48 °N (Figs. 1a and 2a). Atmospheric N deposition was positively correlated with needle N concentration and N:P ratio (Fig. 4a, c, Table S1) and had a negative relationship with needle P concentration (Fig. 4b). MAP, MAT and N deposition interacted negatively in their effect on needle P concentration in the sense that the negative effect of N deposition on needle P concentration and the positive effect on needle N:P ratio became stronger with increasing MAP and MAT (Table S1). The structural analyses show that MAP and MAT had a greater total effect on needle P concentrations and needle N:P ratio than the observed direct
relationships. This was due to their indirect relationships through N deposition (Fig S4). It is also important to note the positive relationships between needle S concentration and throughfall S deposition (Fig. S5) and throughfall N deposition (Fig S6).

**NUTRIENT CONTENT OF THE SOIL IN RELATION TO ATMOSPHERIC N DEPOSITION, CLIMATE, AND NEEDLE N, P AND S CONCENTRATIONS AND N:P RATIOS**

In the subsample of 67 plots of the ICP Forests data base containing long-term series of N bulk deposition, it was possible to compare this data with throughfall N deposition data coming from the ICP Forest. The results from the ICP Forests throughfall N deposition data were similar to those obtained with bulk deposition data (Fig. S7, Table S3) (see supplementary material for detailed information).

Soil solution $\text{NO}_3^-$ concentration, together with MAP and MAT, strongly determined needle N concentration ($R = 0.85$), S concentration ($R = 0.81$) and N:P ratio ($R = 0.8$) (Table S6). $\text{SO}_4^{2-}$ concentration in the soil solution was positively correlated with needle S concentration (Fig. S8, Table S7). $\text{NO}_3^-$ and $\text{SO}_4^{2-}$ concentrations also had positive relationships with $\text{Ca}^{2+}$ and $\text{K}^+$ concentrations in the soil solutions (Table S6). Bulk and throughfall N deposition was negatively correlated with soil Olsen P concentration and positively correlated with soil solution P concentration, total N:Olsen P ratio and soil solution $\text{NO}_3^- : \text{P}$ ratio in the soil organic horizons (Fig. S9, Table S7). Both spatial and non-spatial linear models (OLS and SAR) showed that concentrations of soil Olsen P and soil solution $\text{NO}_3^-$ and the total soil N:Olsen P and soil solution $\text{NO}_3^- : \text{Olsen P}$ ratios were more related to bulk N deposition than to MAP and MAT (Tables S6 and S7). Finally, soil Olsen P and soil solution P concentrations were negatively correlated ($R = -0.37$, $P < 0.03$).

**RELATIONSHIP BETWEEN GROWTH AND MAP, MAT, N DEPOSITION AND NEEDLE COMPOSITION**

Atmospheric bulk and throughfall N deposition had a positive relationship ($R = 0.33$, $P < 0.0001$, and $R = 0.42$ and $P < 0.0001$, respectively) (Fig S5a and S10) with annual absolute BD growth over the period 1995–2005, but not with relative annual BD growth ($R = 0.06$, $P = 0.38$) (data not shown). MAT was positively associated with absolute annual BD growth ($R = 0.36$, $P = 0.0001$) (Fig S5b) over the period 1995–2005, but not with relative annual BD growth (data not shown). There were no significant relationships between MAP and absolute or relative annual BD growth over the period 1995–2005 (data not shown).

Needle nutrient concentrations of N and P were positively associated with absolute annual BD growth, together explaining 23.5% of the total variance of absolute annual BD growth (Fig. S11), whereas only needle P, but not N,
concentration was positively associated with relative annual BD growth (Fig. S11). Needle potassium concentration was also positively associated with absolute annual BD growth over the period 1995–2005 (Fig. S12), but not with relative annual BD growth. Studying the relationships between ‘overall needle elemental composition’ and growth, the PC1 axis scores (explaining a 33.6% of the total variability) of the PCA analysis using needle N, P, K, Ca and Mg concentrations were significantly associated with absolute annual BD growth ($R = 0.57$, $P < 0.0001$) (data not shown) and with relative annual BD growth ($R = 0.30$, $P = 0.001$) (data not shown). This analysis showed that needle N, P and K concentrations were positively correlated with growth, whereas Ca and Mg tended to be negatively correlated. These results thus confirm the trends observed in the univariate relationships.

**EFFECTS OF SOIL AND TEMPORAL TRENDS**

The comparison of 11 plots with podzols with 19 plots with arenosols showed very few differences (Table S8). Only needle P concentrations were significantly higher in podzols than in arenosols.

No temporal trend in N and P concentrations were observed, but needle S concentration tended to decrease significantly in the period 1994–2007 (Fig. 6).
**Discussion**

**RELATIONSHIPS BETWEEN MAP, MAT AND ATMOSPHERIC N DEPOSITION, NEEDLE N AND P CONCENTRATIONS AND GROWTH**

Among the environmental variables studied, MAP and N deposition were the variables most strongly related to needle N concentrations. The highest needle N concentrations, at 45–55°N, occurred in highly industrialized countries, such as the United Kingdom, the Netherlands, Germany and Poland, in line with the highest rates of N deposition. Increasing N deposition generally stimulates plant growth and net primary production (Bedison & McNeil 2009; Krause et al. 2012), a pattern that also holds globally (Fernández-Martínez et al. 2014). Consistent with this, in Europe we have observed a positive relationship between N deposition and absolute but not relative growth of *P. sylvestris*. We have also observed a nutrient imbalance in needles and soils due to N deposition in the same period. N deposition can exceed the requirements of forest growth and lead to destabilization and forest decline (Magill et al. 2004; Sutton et al. 2011). In general, long-term studies show an initial enhancement of tree growth that with time can be followed by a tree decline linked to soil nutrient imbalances, increased cation leaching from soil and changes in soil pH (Högberg et al. 2006; Juknys et al. 2014). Atmospheric N deposition could thus have a positive fertilizing effect on the growth of *P. sylvestris*, but this effect could be counteracted in a future when other resources, such as P, become limiting. Most European areas of this study have a previous long-term period of atmospheric N deposition (Sutton et al. 2011), which can explain the observed lower needle P concentrations and higher N:P ratios in forests with higher levels of N deposition.

However, no general temporal trend in needle N and P concentrations was observed in the period 1994–2007 (Fig. 6). In this period, whereas absolute growth was positively associated with both needle N and P concentrations, relative growth was only related to needle P concentration. The data thus suggest that P is as much or even more limiting than N across Europe. In this regard, increased growth and size of individual *P. sylvestris* trees has been associated with higher needle P concentrations and lower N:P ratios (Portsmuth et al. 2005). The lower P concentrations and higher N:P ratios could hinder the growth and regeneration of *P. sylvestris*, a moderately fast-growing species. In younger *P. sylvestris* stands, with higher relative growth rates, the observed negative relationship between N deposition and needle P concentration, especially at medium and high latitudes, could become greater. This observed higher association between P (rather than N) and relative growth rates that is consistent with the growth rate hypothesis (GRH) (Sterner & Elser 2002), which predicts a pivotal capacitating role of P in protein synthesis to sustain intense growth rates. The relationships between foliar N concentrations and protein synthesis rates can, however, be complex and not necessarily linear (Matzek & Vitousek 2009). These differences in needle P concentrations and N:P ratios were especially large where high levels of N deposition coincided with both high MAT and MAP. Hence, larger nutrient imbalances due to N deposition can be expected at sites with higher productivity, where P is depleted faster. The optimum MAT and MAP conditions for *P. sylvestris* are most likely met in central Europe, coinciding with the centre of the species’ distribution and also with the higher levels of N deposition.

All these results further suggest that P could become increasingly limiting in areas with elevated N deposition. These results are in agreement with previous studies, suggesting an increase in P limitation in several forested areas at central latitudes in Europe characterized by high N deposition (Harrison et al. 1999). An increase in N deposition can stimulate soil P immobilization in forested areas by the enhanced uptake of P by the soil food web (Piatek et al. 2009); several studies have observed increased P limitation with high and continuous N loading (Phoenix et al. 2004; Gradowski & Thomas 2008). Various plant species can respond to increases in N supply, improving P absorption, enhancing P uptake or increasing the capacity to reabsorb P (Phoenix et al. 2004; Fujita et al. 2010). When N loading surges, plants and soil organisms enhance their mechanisms to increase P uptake, such as a higher synthesis of phosphatases, but these mechanisms do not prevent an increase in the N:P ratio in the long term (Penuelas et al. 2013). These results also suggest that climatic gradients or atmospheric N deposition can have an asymmetric impact on the N and P status of plants, with a larger impact on the N:P ratio than on the individual N and P status. Despite the significant role of N deposition and the higher needle N concentrations in central Europe, we have also observed a trend of lower N:P ratios at high latitudes relative to medium and low latitudes, suggesting a limiting role of N in northern *P. sylvestris* populations (Oleksyn et al. 2003). In fact, these results are consistent with the soil age hypothesis which claims that soils in northern ecosystems are younger because the glaciers retreated only recently, and the young soils that then developed are richer in P and poorer in N compared to older soils at lower latitudes. Soils generally tend to evolve from N limitation to N productivity to P limitation over time (Walker & Syers 1976; Chadwick et al. 1999). This trend was also observed in this study, as *P. sylvestris* forests at lower latitudes, such as those on the Iberian and Balkan Peninsulas, tended to have the highest N:P ratios, whereas the opposite was observed in Scandinavian *P. sylvestris* forests at high latitudes. Despite the considerable levels of atmospheric P deposition from the Saharan and Turkish deserts (Mahowald et al. 2008; Camarero & Catalan 2012), this phenomenon did not increase the needle P concentrations of *P. sylvestris* forests in either of these two peninsulas. However, the low values of needle P concentrations observed in the lower latitude area of distribution of *P. sylvestris* were very probably related to *P. sylvestris*...
forest decay under the present scenario of increased drought frequency and intensity in the Mediterranean area (Sanchez-Salguero et al. 2012; Poyatos et al. 2013), where strong positive relationships between needle nutrient concentrations and MAP have been also observed (Sardans, Rivas-Ubach & Peñuelas 2011).

The average N:P ratio across the 2245 P. sylvestris stands included in our study was $10.8 \pm 0.06$. Other studies of European forests have reported higher N:P ratios, even in coniferous forests. For example, Sardans, Rivas-Ubach & Peñuelas (2011) reported an average N:P ratio of 12.7 $\pm$ 0.4 among more than 1000 conifer stands in Catalonia (Sardans, Rivas-Ubach & Peñuelas 2011). In the sites with values of N deposition of 2-5 kg N ha$^{-1}$ year$^{-1}$, the needle N:P ratio was 8.7 on average, whereas in sites with N deposition of 20 N ha$^{-1}$ year$^{-1}$ the needle N:P ratio was 12 on average, showing that despite a certain level of leaf N:P plasticity, the values of N:P tend to be low N:P ratios. Low N:P ratios in photosynthetic tissues are often associated with high growth rates (Sterner & Elser 2002; Elser et al. 2003; Peñuelas & Sardans 2009; Rivas-Ubach et al. 2012). When studying several different groups of plant together, leaf N:P ratios range from 5 to 65 with a mean of 28-30 (Elser et al. 2000, 2010), and thus, the observed average N:P ratio of 10.8 $\pm$ 0.06 is in agreement with P. sylvestris being a relatively fast-growing species occupying an early stage of forest succession (Picón-Cochard, Coll & Balandier 2006; Marozas, Racinskas & Bartkevicius 2007).

Continuous loadings of N in P. sylvestris forests due to N deposition were associated with an increase in the N:P ratio as a result of increasing N concentrations, but also due to decreasing P concentrations. The percentage of variance of N and P concentrations explained by N deposition, although significant, was low, and several other factors, such as soil type and characteristics, could explain a large part of the variance found in the statistical analyses. Overall, variance of N and P needle concentrations was particularly high in the analyses using the larger network of sites when compared with using only data from the ICP Forests data base. This is not surprising since although the analytical techniques used were comparable in all data sources, the ICP Forest data base’s sampling and analytical processes have standard-ized protocols which are distinct to those used in the Spanish forest inventory or to methods used to collect other data coming from very different studies. Although this variance was greatest in analyses using the larger network of sites, it was still relatively high when using only the ICP Forests data set. The results strongly suggest that one of the causes underlying this high variance (not explained by climate and atmospheric N deposition) was soil variability. There were positive relationships between soil solution nitrate and sulphate and needle N and S concentrations ($R = 0.73$, $P < 0.0001$ and $R = 0.71$, $P < 0.0001$, respectively). Unfortunately, set of plots with available soil type information was too small to allow a deeper analysis of this matter. All in all, the negative relationships between N throughfall and bulk deposition with needle P concentration would likely have a negative impact on growth of this fast-growing tree species. These results suggest that N deposition could be particularly negative for fast-growing species and that the N:P ratio can be a possible indicator of the effects of atmospheric N deposition on plant growth or vitality, although more research is needed to confirm its use.

**RELATIONSHIPS BETWEEN MAP, MAT AND ATMOSPHERIC N DEPOSITION AND SOIL CHEMISTRY: ARE THEY RELATED TO SHIFTS IN NEEDLE N, P AND S CONCENTRATIONS AND N:P RATIOS?**

This study shows that N deposition leads to nutrient imbalances in European P. sylvestris ecosystems, especially in the soils. The positive relationships of N deposition with needle N concentration and of N:P ratio with soil solution NO$_3^-$ concentration strongly suggest that European P. sylvestris forests have been a large sink of N but tend to be N saturated in areas with higher N deposition. This is in line with most studies of natural ecosystems under high levels of long-term N deposition with N saturation of the soils (Aber et al. 2003; Pilkington et al. 2005). The high concentrations of NO$_3^-$ in the soil solution may suggest that P. sylvestris forests under higher rates of N deposition are unable to immobilize all of the deposited N, thus favouring N losses by leaching and/or surface run-off. These results are in agreement with previous studies observing increases in soil solution NO$_3^-$ concentration and in N run-off and leaching in temperate forests under high levels of N deposition, even when gaseous N losses during nitrification and denitrification are frequently enhanced (Stuines & Kjonaas 1998; Pilkington et al. 2005; Fatemi et al. 2012).

Our study further revealed that N deposition was negatively correlated with the concentration of extractable soil Olsen P, representing P available to plants, and positively with the soil solution NO$_3^-$:P ratio. Our results also showed that N deposition was positively correlated with soil solution P concentration. Olsen P concentration was therefore negatively correlated with soil solution P concentration, thus suggesting a large displacement of exchangeable P (fixed in labile bonds within the soil complex) towards the soil solution. While elevated N deposition increased soil solution P concentrations, deposition increased soil solution NO$_3^-$ concentrations even more greatly, thus increasing the soil solution NO$_3^-$:P ratio. As a consequence of N deposition, the reserve of soil P available to plants thus gives the impression of depletion, while N and P loadings to run-off increase: this may account for the high needle N:P ratio in P. sylvestris and the high soil and soil solution N:P ratios observed under high levels of atmospheric N deposition. These high ratios under high levels of atmospheric N deposition thus show that P. sylvestris trees are unable to maintain their needle N:P concentrations...
ratios and P concentrations, despite the fact that the available data would suggest enhanced P uptake from soils.

The results of this study are consistent with several studies in northern and central Europe and North America, observing that N deposition has increased N:P ratios in soils and plants (Bragazza et al. 2004; Fujita et al. 2010). Our results thus support the premise that the continuously increasing N:P ratios due to N inputs into the biosphere, although geographically extremely variable, now threaten to provoke a widespread N:P imbalance in natural ecosystems (Peñuelas et al. 2012, 2013; Sardans, Rivas-Ubach & Peñuelas 2012b).

Positive relationships have been observed among the soil solution concentrations of K\(^{+}\), Ca\(^{2+}\), SO\(_{4}\)\(^{2-}\) and NO\(_{3}\)\(^{-}\) and N deposition. Increased concentrations of Ca\(^{2+}\) in soils have also been observed in other studies and are associated with soil acidification under high N and S deposition, whereas protons are exchanged with Ca\(^{2+}\) or neutralized by weathering processes (Rustad et al. 1993; Verstraeten et al. 2012). These observed increases in Ca\(^{2+}\), NO\(_{3}\), K\(^{+}\) and SO\(_{4}\)\(^{2-}\) concentrations suggest that losses of these nutrients in run-off might increase in a manner which is proportional to the level of N deposition. The increase in soil solution K\(^{+}\) concentration in stands with high N loadings also suggests shifts in the K cycle and N:K ratios. Although shifts in K cycling and the stoichiometric relationships of K with N and P in terrestrial ecosystems across natural gradients or in response to global change have been studied very little, it is known that they can change across climatic gradients (Sardans et al. 2012c) and especially in plants subjected to drought (Rivas-Ubach et al. 2012).

These results thus demonstrate that both climate and atmospheric N deposition can modify plant nutrient stoichiometry. The documented shifts in the N:P ratio and changes in other nutrient concentrations in P. sylvestris along abiotic gradients is a suitable contribution for the discussion of the limits of stoichiometric homeostatic capacity and flexibility (Elser et al. 2000). Increasingly imbalanced inputs will require substantial upregulation of the mechanisms of homeostasis and flexibility and their corresponding energetic costs. An organism’s capacity to adapt to environmental shifts may be exceeded because this cost decreases growth and reproductive capacity (Borsma & Elser 2006). Further investigation should focus on the responses of P. sylvestris to continued N loading and changes in the competitive advantage of other forest species, the occurrence of diseases, tree mortality or seedling recruitment rates.

Scaling relationship between needle N and P concentrations

In this study, needle N concentration scaled at 0.498 relative to P concentration (log [N] = 0.556 + 0.498 log [P]). This result is consistent with previous studies showing that foliar N:P ratio decreases when foliar P (and also N) concentration increases (Niklas et al. 2005; Reich et al. 2010), and is also consistent with what is expected according to the GRH (Sterner & Elser 2002).

We observed that the proportionally higher increase in P led to a decrease in the N:P ratio when both foliar N and P concentrations increased (Fig. 3). This relationship suggests that N:P ratios tend to decrease when the productive capacity of plants rises. A lower N:P ratio has been associated with a higher growth rate capacity, especially when the concentrations of both N and P are high (Vrede, Andersen & Hessen 1999; Vrede et al. 2004).

Temporal trends

Our results also suggest that needle S concentrations in European P. sylvestris forests have decreased over the last two decades, while needle N and P concentrations have remained more or less stable. These findings are consistent with the general patterns of change in atmospheric N and S deposition in Europe over the past decades: a modest reduction in N emissions associated with agriculture (Sutton et al. 2011) and a dramatic reduction of S emissions since the early 1980s following a scaling down of coal combustion and the implementation of policies to improve air quality (Eurostat 2009). Moreover, the efficiency of environmental policies for reducing N emissions varies among European regions, with some countries still increasing emissions in recent decades.

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Data accessibility

The data do not belong to the authors. We have cited the corresponding references and data sources (TRY, ICP, Ecological Forest Inventory of Catalonia, Third Spanish National Forest Inventory and Web of science) in the text.

References


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Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. Results of the GLM analyses without and with spatial correction (upper and lower cells), with needle N and P concentrations and N:P ratio as dependent variables and climatic (MAP and MAT) variables and N deposition (EMEP model) and their significant interactions as independent variables, in the studied 2245 P. sylvestris stands across Europe.

Table S2. Results of the GLM without and with spatial correction (upper and lower cells), with needle N, P, and S concentrations and N:P ratio as dependent variables and climatic (MAP and MAT) variables and N deposition (EMEP model) and their signif-
N deposition affects Scots pine stoichiometry

Results of the GLM without and with spatial correction (upper and lower cells), with needle N, P, and S concentrations and N:P ratio as dependent variables and climatic (MAP and MAT) variables and throughfall N deposition (ICP) and their significant interactions as independent variables, in 167 European Pinus sylvestris stands monitored by the ICP Forests Level II programme.

Table S3. Results of the GLM without and with spatial correction (upper and lower cells), with needle N, P, and S concentrations and N:P ratio as dependent variables and climatic (MAP and MAT) variables and throughfall N deposition (ICP) and their significant interactions as independent variables, in 55 European P. sylvestris stands monitored by the ICP Forests Level II programme.

Table S4. Results of the GLM analyses without and with spatial correction (upper and lower cells), with soil chemical traits as dependent variables and climatic (MAP and MAT) variables and N deposition (EMEP model) and their significant interactions as independent variables, in 54-67 European P. sylvestris stands monitored by the ICP Forests Level II programme.

Table S5. Results of the GLM analyses without and with spatial correction (upper and lower cells), with soil chemical traits as dependent variables and climatic (MAP and MAT) variables and throughfall N deposition (ICP) and their significant interactions as independent variables, in 36 European P. sylvestris stands monitored by the ICP Forests Level II programme.

Table S6. Results of the GLM without and with spatial correction (upper and lower cells), with needle N, P, and S concentrations and N:P ratios as dependent variables and climatic (MAP and MAT) variables and soil-solution NO$_3^-$ concentrations and their significant interactions as independent variables, in 67 European P. sylvestris stands monitored by the ICP Forests Level II programme.

Table S7. Correlations among the elemental and ionic concentrations and ratios of needles and soil solutions in 67 European P. sylvestris stands monitored by the ICP Forests Level II programme.

Table S8. Comparison (one way ANOVA) of the main needle and soil studied chemical variables between plots with podzols (11) and plots with arenosols (19).

Fig. S1. Location of the 67 P. sylvestris ICP Forests Level II plots with soil data.

Fig. S2. Maps of bulk N-NO$_3^-$ and N-NH$_4^+$ deposition (kg ha$^{-1}$) corresponding to the projections for 2011 from EMEP model.

Fig. S3. Latitudinal gradient of bulk atmospheric N deposition (average from 1991-2006, from EMEP model) in P. sylvestris forests across Europe.

Fig. S4. Structural equation modeling results of the direct and indirect relationships (by bulk N deposition) of MAP and MAT on needle N and P concentrations and N:P ratio data for 2245 forest plots of P. sylvestris spread across its entire range of distribution.

Fig. S5. Relationship of needle S concentration with N throughfall deposition (from ICP forests model) in 52 European P. sylvestris stands monitored by ICP Forests (Level II). Data are an annual average obtained in the period 1995-2005.

Fig. S6. Relationship of needle S concentration with throughfall N deposition in 52 European P. sylvestris stands monitored by ICP Forests (Level II).

Fig. S7. Relationships of the NO$_3^-$ (a), NH$_4^+$ (b), total N (c) and SO$_4^{2-}$ (d) deposition observed values for throughfall deposition (ICP Forests Level II) with the expected values by the EMEP model within the period from 1995 to 2011 in 98 different P. sylvestris forests along 18 European countries.

Fig. S8. Relationships of needle N (a) and P (b) concentrations and S concentration (c) and N:P ratio (d) with soil-solution NO$_3^-$ concentration and of needle S concentration with soil-solution SO$_4^{2-}$ concentration (e) in 67 European P. sylvestris stands monitored by ICP Forests (Level II).

Fig. S9. Relationships of soil Olsen P concentration (a), total soil N:Olsen P ratio (b), soil-solution P concentration (c), and soil-solution NO$_3^-$:P ratio (d) with N deposition in 55 European P. sylvestris stands monitored by ICP Forests (Level II).

Fig. S10. Relationships of annual absolute basal diameter (BD) growth with total annual throughfall N deposition (from ICP Forests data).

Fig. S11. 3D representation of the relationship between absolute DB annual growth (a) and relative BD annual growth (b) with needle N and P concentrations.

Fig. S12. Relationship between absolute BD annual growth (cm) and needle K concentration (mg g$^{-1}$).

Fig. S13. Relationships of needle N concentrations (a), P needle concentrations (b), needle N:P ratio (c) with N deposition in 67 European P. sylvestris stands monitored by ICP Forests (Level II).

Fig. S14. Relationships of soil-solution NO$_3^-$ (a), K$^+$ (b), and SO$_4^{2-}$ (c) concentrations with bulk N deposition (EMEP model) in 67 European P. sylvestris stands monitored by ICP Forests (Level II).

Fig. S15. Relationships of soil-solution NO$_3^-$ (a), K$^+$ (b), SO$_4^{2-}$ (c) and Olsen_P (d) concentrations, and with throughfall N deposition (ICP Forests (Level II) data) in 41 (36 for P-Olsen) European P. sylvestris stands monitored by ICP Forests (Level II).