

1 **Foliar and soil concentrations and stoichiometry of N and P across European**
2 ***Pinus sylvestris* forests: relationships with climate, N deposition and tree**
3 **growth.**

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27 Running head: N deposition affects Scots pine stoichiometry

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Post-print of Sardans, Jordi et al. «Foliar and soil concentrations and stoichiometry of N and P across European *Pinus sylvestris* forests: relationships with climate, N deposition and tree growth.» in Functional Ecology (Wiley). Vol 30, Issue 5 (May 2016), p. 676-689. The final version is available at DOI 10.1111/1365-2435.12541

29 **Summary**

30 1. This study investigated the factors underlying the variability of needle and soil
31 elemental composition and stoichiometry and their relationships with growth in *Pinus*
32 *sylvestris* forests throughout the species' distribution in Europe by analyzing data from
33 2245 forest stands.

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

40 3. Atmospheric N deposition was positively correlated with soil-solution NO_3^- , SO_4^{2-} , K^+ ,
41 P, and Ca^{2+} concentrations, the soil-solution NO_3^- :P ratio, total soil N, and the total soil
42 N:Olsen P ratio, and negatively correlated with soil Olsen P concentration.

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

49 5. These results thus indicate a tendency of European *P. sylvestris* forests to store N in
50 trees and soil in response to N deposition and unveil a trend toward increased nutrient
51 losses in runoff as a consequence of higher soil-solution N concentrations. Overall, the
52 data show increasing ecosystem nutrient imbalances with increasingly limiting roles of
53 P and other nutrients such as K in European *P. sylvestris* forests, especially in the
54 center of their distribution where higher levels of N deposition are observed. Thus,
55 although the data show that N deposition has had an overall positive effect on *P.*
56 *sylvestris* growth, the effect of continuous N deposition, associated with decreasing P

57 and K and increasing N:P in leaves and in soil, may in the future become detrimental
58 for the growth and competitive ability of *P. Sylvestris* trees.

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

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85 **Introduction**

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

98 These studies have typically been carried out as analyses of data from a large
99 number of species. In contrast, analyses of stoichiometric differences within single
100 species are much less common. Khang *et al.* (2011) studied the differences in N and P
101 concentrations and the N:P ratio in the needles of *Picea abies* in several European
102 countries and observed higher N and P concentrations in the central part of the tree's
103 distribution, suggesting that higher needle N and P concentrations are associated with
104 the optimum climatic conditions for biomass production. In line with this, both *P. abies*
105 and *Pinus sylvestris* have higher growth and survival rates in the centers of their
106 distributions (Sykes & Prentice 1995). Anthropogenic eutrophication of ecosystems as
107 a result of atmospheric N deposition and climate change can alter plant N and P
108 concentrations and the N:P ratios of soils, water, and organismic tissues (Elser *et al.*
109 2010; Sardans *et al.* 2012a; Penuelas *et al.* 2012, 2013). A growing number of studies
110 have reported an increase in the N:P ratios of aquatic and terrestrial plants in response
111 to increasing N loadings (Sardans *et al.* 2012a; Peñuelas *et al.* 2012, 2013). N
112 deposition can also affect soil properties, including N concentrations (Rustad *et al.*

113 1993; Verstraeten *et al.* 2012) and cause nutrient imbalances (Fujita *et al.* 2010),
114 which have not yet been globally assessed (Peñuelas *et al.* 2012, 2013; Sardans,
115 Rivas-Ubach & Peñuelas 2012b). Atmospheric N deposition accelerates the N cycle,
116 increasing N availability, while other essential nutrients (P, K, Ca, Mg) may become
117 limiting factors (Sutton *et al.*, 2011). Changes in plant N:K ratios caused by N
118 deposition have been observed in European *Calluna vulgaris* heathlands (Britton *et al.*
119 2008; Rowe *et al.* 2008). Sulfur (S) deposition is frequently associated with N
120 deposition (van Dijk *et al.* 2012; Aherne & Posch 2013) changing soil and plant
121 elemental composition and stoichiometry (Robroeeek *et al.* 2009; van Dijk *et al.* 2012).
122 This further affects the demographic, phenotypically plastic, and adaptive responses of
123 plants (Carnicer *et al.* 2014) so S and N deposition are usually studied together to
124 analyse the impacts of human-driven global change on the plant-soil system.

125 Stoichiometric flexibility has been defined as the ability of organisms to adjust
126 their elemental ratios while maintaining constant functions (Sistla & Schimel 2012).
127 This flexibility can act at different scales, from individual organisms at the physiological
128 level (e.g. reallocation and changes in uptake strategies) and ontological level to
129 communities and ecosystems (e.g. changes in species composition). Most plants have
130 some degree of stoichiometric flexibility (Güsewell 2004), but most terrestrial plant
131 species are less stoichiometrically flexible than plankton are, and some even exhibit
132 considerable stoichiometric homeostasis. Little is known, however, about the capacity
133 of terrestrial plant species to have flexible stoichiometries along their natural ranges of
134 distribution in response to natural and anthropogenic gradients. Hence, investigating
135 the degree to which terrestrial plant species adjust their internal stoichiometries in
136 response to environmental changes is necessary to obtain knowledge of stoichiometric
137 flexibility and its ecological significance. These studies should allow us to understand
138 the role of stoichiometric flexibility, as opposed to stoichiometric homeostasis, and its
139 relationship with ecological characteristics of species, such as growth strategy,
140 capacity to adapt to environmental changes, and nutrient use efficiency. Moreover, as

141 recent reviews suggest, the role played by stoichiometric flexibility in linking micro- to
142 macro-scale biogeochemical cycles in a changing world requires investigation (Sistla &
143 Schimel 2012). In this sense, anthropogenic processes, such as those that increase N
144 deposition, can also affect plant stoichiometry both directly and by interacting with
145 other environmental variables such as climatic variables. Thus, we hypothesized that
146 atmospheric N deposition can change needle elemental composition in *P. sylvestris*
147 forests across Europe and that this, at least in part, is related to the effects of N
148 deposition on soil chemistry.

149 *P. sylvestris* is one of the most important forest species in Eurasia, with a broad
150 distribution extending from Spain to Siberia (Cheddadi *et al.* 2006). This distribution
151 across Europe offers a unique opportunity to study the relationships of soil and plant
152 nutrient concentrations and stoichiometry with climate, atmospheric N deposition, and
153 plant growth. A previous study, using a data set of 195 stands of *P. sylvestris* across
154 Europe, reported a trend toward increased needle N concentrations with latitude
155 (Oleskyn *et al.* 2003). Some of these data, however, were obtained in the 1960s,
156 before the dramatic increase in N deposition due to human activities (Prietzl & Stetter
157 2010). The considerable climatic gradients (mean annual precipitation (MAP), 335-
158 1768 mm yr⁻¹; mean annual temperature (MAT), -2.1-16.1 °C) and the variation in N
159 deposition (0.55-27.0 kg N ha⁻¹) across the distribution of *P. sylvestris* make this
160 species an adequate model to test the links between these variables and their
161 interactions, growth, and the soil-plant stoichiometric response. Furthermore, we
162 aimed to investigate whether the effects of climatic variables and N deposition on *P.*
163 *sylvestris* N:P ratio can be due, at least partially, to the indirect effects of changing soil
164 conditions. We hypothesized that N deposition could have contributed to imbalanced
165 elemental composition of soil and foliage in *P. sylvestris* forests across Europe, and
166 further that this could probably be linked to changes in growth patterns resulting from
167 the balance between the positive fertilization effect and the negative nutrient
168 imbalance effect.

169 In this study, we investigated the needle-soil elemental composition and
170 stoichiometric flexibility of *P. sylvestris* forests and their imbalances in response to
171 climatic gradients and atmospheric N deposition. We also probed for additional
172 relationships of those variables with *P. sylvestris* growth by analysing a compiled data
173 set of 2245 stands of *P. sylvestris* throughout Europe, obtained mainly from 1990 to
174 2006. The specific objectives of this study were to discern: (i) the patterns of needle N,
175 P, K, and S concentrations and N:P ratio throughout the European distribution of *P.*
176 *sylvestris*, (ii) the allometric relationships between needle N and P concentrations that
177 could possibly affect N:P ratios, (iii) the impacts of climate and N deposition on needle
178 and soil N, P, K, Ca, Mg and S concentrations and N:P ratio, (iv) the effects of temporal
179 trends of atmospheric N deposition on needle N, P, K, Ca, Mg and S concentrations,
180 and (v) the effects of N deposition and needle nutrient concentrations on growth of *P.*
181 *sylvestris*.

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197 **Material and Methods**

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199 EUROPEAN DATA FOR NEEDLE AND SOIL NUTRIENT CONCENTRATIONS

200 Data for needle N and P concentrations of Scots pine (*P. sylvestris*) were obtained
201 from the web of science, <http://apps.webofknowledge>, the ICP Forests monitoring
202 program (International Co-operative Programme on Assessment and Monitoring of Air
203 Pollution Effects on Forests, operated under the UNECE Convention on Long-range
204 Transboundary Air Pollution, <http://icp-forests.net/>, Fisher *et al.* 2012), the TRY
205 database (Kattge *et al.* 2011), the Ecological Forest Inventory of Catalonia (Gracia *et*
206 *al.* 2004), and the Third Spanish National Forest Inventory (Villanueva 2005). The data
207 only included sites where *P. sylvestris* was a pure stand or the dominant tree species.
208 The data were harmonised by selecting data from studies that determined N
209 concentration by Kjeldahl and/or dry combustion, used a Carlo Erba elemental
210 analyser, and determined P, K, Ca, Mg and S concentrations by atomic spectrometric
211 methods. Needle N:P ratios were based on mass. We obtained needle data for 2245
212 forest plots of *P. sylvestris* spread across its entire range in Europe with samples
213 collected between 1990 and 2006 (Fig. 1). Additionally, data for needle N, P, and S
214 concentrations for the period 1994 to 2007 in the ICP Forests database were available
215 for 90 *P. sylvestris* plots distributed across twelve European countries. These data
216 were used to study the temporal shifts in needle nutrient concentrations. Data for
217 needle N, P, K, Ca and Mg concentrations from the ICP Forests database were also
218 available for 119 plots from 16 countries, and annual absolute and relative basal
219 diameter (BD) growth were available for 189 plots from 18 countries in the period 1995-
220 2005. To compare growth and needle elemental composition with climate and N
221 loadings, we also collected the climate and N loadings (throughfall N deposition and
222 MAP from ICP) for 82 of the 189 plots from which growth data were available for the
223 same plots and periods. To compare needle elemental composition with climate and N
224 loadings (throughfall N deposition and MAP from ICP) we also collected throughfall and

225 MAP data from 55 of the 119 plots from which needle elemental composition data were
226 available for the same plots and periods. Absolute DB growth was calculated as the
227 average stand diameter annual growth, whereas diameter was measured at breast
228 height and the relative DB growth is the absolute stand diameter growth relative to the
229 initial stand BD.

230 We also used the soil solution chemistry (NO_3^- , K^+ , Ca^{2+} , and SO_4^{2+} of organic
231 horizons) of 67 *P. sylvestris* plots (Fig. S1) monitored under the ICP Forests Intensive
232 Forest Monitoring (Level II) from 1995 to 2005; P concentration in soil solution of the
233 organic horizons was available for 54 of these 67 plots. In these 67 plots needle S, N
234 and P and N:P ratio data were available for the same plots and periods and we were
235 able to analyse the relationship between soil NO_3^- and needle elemental composition.
236 N throughfall and MAP data were available for the same plots and periods in 4167 plots
237 (from ICP Forests Intensive Forest Monitoring). Data for total soil N, Ca, K, and Olsen
238 P (a proxy for soil P available to plants) concentrations were available for 57 plots
239 monitored under the ICP Forests Intensive Forest Monitoring (Level II) from 1995 to
240 2005. Data on soil variables and N throughfall and MAP from ICP were available in 36
241 plots for the same plots and periods. We used these data to analyse the relationships
242 between soil chemistry and climate (WorldClim database and ICP Forests), and N
243 deposition (from EMEP and ICP Forests). These data were used to study the
244 relationships between absolute and relative annual BD growth, climate (from WorldClim
245 database and using MAP from the same plots), atmospheric N deposition (from EMEP
246 and from ICP Forests) and needle elemental composition. We had soil taxonomic
247 information for 32 plots: most soils were podzols (11) and arenosols (19), and only one
248 plot had umbrisols and another cambisols. We have analysed the differences in
249 needle elemental concentration and ratios and in some available soil variables between
250 podzols and arenosols. All georeferenced data were processed using MiraMon 6.0
251 (Pons 2009). All commented statistical analyses with the corresponding data sources,
252 and Figures and Tables showing results are presented in Table 1.

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254 CLIMATIC AND N-DEPOSITION DATA

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

261 Data for atmospheric N deposition were obtained from the European Monitoring
262 and Evaluation Programme (EMEP) of the Convention on Long-range Transboundary
263 Air Pollution (CLRTAP). The EMEP MSC-W chemical-transport model (Simpson *et al.*
264 2012) has been developed to estimate regional atmospheric dispersion and deposition
265 of acidifying and eutrophying compounds (S, N), ground-level ozone (O₃), and
266 particulate matter (PM_{2.5}, PM₁₀). This study used data for total atmospheric N and S
267 deposition estimated annually for the period 1990-2006 with EMEP model rv3.8.1 over
268 Europe using a grid size of 50 km × 50 km (EMEP, 2011). In Fig. S2 are shown the N-
269 NO₃⁻ and NH₄⁺ in bulk deposition (kg ha⁻¹) of the year 2011 in Europe.

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271 DATA ANALYSES

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

278 To check the relationships between all studied variables (climatic, atmospheric
279 N deposition, needle, and soil variables), we used multiple correlations performed with
280 STATISTICA 6.0 (StatSoft, Inc. Tule, OK, USA). In the multiple correlations we applied

281 the Bonferroni correction to adjust confidence intervals and P values. We used the R
282 package mgcv (Wood 2000) for generalized additive models to smooth the trends of
283 [N], Log [P], Log N:P, and Log N deposition with latitude by means of thin plate spline
284 regressions. We also conducted principal component analyses to thereafter correlate
285 PC scores, as proxies of overall needle elemental composition, with growth.

286 We applied general linear models (GLMs) to analyse the relationships of climatic
287 variables (MAP and MAT) with atmospheric N deposition and their interactions on
288 needle N, P, K, and S concentrations and N:P ratios and on soil and soil solution
289 nutrient concentrations and stoichiometries. Needle N concentration was normally
290 distributed, whereas P concentration and the N:P ratio were not. To normalize their
291 distributions, these variables were log-transformed in the analyses. In the models used
292 to analyse the relationships between MAP, MAT, and N deposition with the needle and
293 soil variables and growth, as well as the relationships between needle variables and
294 growth, the degree of spatial autocorrelation in the residuals was assessed using
295 Moran's I correlograms and by plotting spatial maps of the distribution of residuals
296 (following Carnicer et al. 2011). To assess the level of significance at each distance
297 class in the Moran's I correlograms, we computed 1000 permutations using the resamp
298 argument in the correlog function (ncf package, R software). When significant
299 autocorrelation in the residuals was detected in ordinary least squares (OLS) models,
300 we applied spatial statistical modelling methods (spatial simultaneous autoregressive
301 (SAR models) to remove, or at least reduce, the spatial autocorrelation in the residuals
302 of the models. In these models, we used MAP, MAT, and total atmospheric N
303 deposition as continuous independent variables. We used a factorial design, but in the
304 final models we only maintained the interactions among the significant independent
305 variables.

306 To detect the possible associations between climate traits and needle elemental
307 composition via direct relationships but also by indirect relation through N deposition
308 we used Structural Equation Modelling (SEM). We fitted the different models using the

309 sem R package (Fox *et al.* 2013) and achieved the minimum adequate model using the
310 AIC. Standard errors and the level of significance (*P*-value) of the total, direct and
311 indirect relationships were calculated using the bootstrap (with 1200 repetitions)
312 technique (Davison *et al.* 1986; Mitchell-Olds 1986).

313 We used 1027 records from ICP Forests data of NO_3^- , NH_4^+ , SO_4^{2-} and total N in
314 throughfall of *Pinus sylvestris* forests over the period of 1995 to 2011 in 98 different
315 sites in 18 European countries. We used this data to analyse the relationships between
316 these throughfall data from ICP Forests and the corresponding data (in the same sites
317 and years) of bulk deposition from the EMEP model. In this way, we analysed whether
318 or not EMEP data accurately determined, without under- or overestimation, the
319 loadings of N and S in forest soils.

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

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337 **Results**

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339 NEEDLE N AND P CONCENTRATIONS AND N:P RATIOS ACROSS EUROPE

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341 Both needle N and P concentrations have significant variability within the latitudinal
342 gradient, but both were higher in the central latitudes within the area of distribution. The
343 thin plate spline regressions showed maximum needle N concentrations of 2.34 ± 0.1
344 (% DW) at 48° N (Figs. 1a and 2a) whereas needle P concentrations increased
345 beginning from south Europe to mid latitudes and then had relatively constant values
346 with increasing latitude (Fig 2b).

347 Needle N:P ratios, however, tended to decrease from low to high latitudes (Figs.
348 1b and 2c). MAP, MAT, and total atmospheric N deposition ranged from 733 to 1768
349 mm yr^{-1} , from -2.1 to 16.1°C , and from 0.55 to $27 \text{ kg ha}^{-1} \text{ yr}^{-1}$, respectively (Hijmans *et*
350 *al.*, 2005; Simpson *et al.* 2012). On a log-log scale, needle N concentrations scaled at
351 0.423 relative to needle P concentrations (Fig. 3a), indicating that P concentrations
352 increased proportionally more than needle N concentrations when both concentrations
353 increased (Fig. 3b).

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355 RELATIONSHIPS BETWEEN NEEDLE N, P AND S CONCENTRATIONS AND N:P
356 RATIO WITH ATMOSPHERIC N DEPOSITION AND CLIMATE

357

358 Within the European distribution of *P. sylvestris* forests, maximum atmospheric N
359 deposition occurred near 52°N (Fig. S3), near the observed latitude of maximum
360 needle N concentration of 48°N (Figs. 1a and 2a). Atmospheric N deposition was
361 positively correlated with needle N concentration and N:P ratio (Figs. 4a and 4c, Table
362 S1) and had a negative relationship with needle P concentration (Fig. 4b). MAP, MAT,
363 and N deposition interacted negatively in their effect on needle P concentration in the
364 sense that the negative effect of N deposition on needle P concentration and the

365 positive effect on needle N:P ratio became stronger with increasing MAP and MAT
366 (Table S1). The structural analyses show that MAP and MAT had a greater total effect
367 on needle P concentrations and needle N:P ratio than the observed direct
368 relationships. This was due to their indirect relationships through N deposition
369 (supplementary material Fig S4). It is also important to note the positive relationships
370 between needle S concentration and throughfall S deposition (Figure S5) and
371 throughfall N deposition (Fig S6).

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373 NUTRIENT CONTENT OF THE SOIL IN RELATION TO ATMOSPHERIC N 374 DEPOSITION, CLIMATE, AND NEEDLE N, P AND S CONCENTRATIONS AND N:P 375 RATIO

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

382 Soil solution NO_3^- concentration, together with MAP and MAT, strongly
383 determined needle N concentration ($R = 0.85$), S concentration ($R = 0.81$), and N:P
384 ratio ($R = 0.8$) (Table S6). SO_4^{2-} concentration in the soil solution was positively
385 correlated with needle S concentration (Fig. S8, Table S7). NO_3^- and SO_4^{2-}
386 concentrations also had positive relationships with Ca^{2+} and K^+ concentrations in the
387 soil solutions (Table S6). Bulk and throughfall N deposition was negatively correlated
388 with soil Olsen P concentration and positively correlated with soil-solution P
389 concentration, total N:Olsen P ratio, and soil-solution NO_3^- :P ratio in the soil organic
390 horizons (Fig. S9, Table S7). Both spatial and non-spatial linear models (OLS and
391 SAR) showed that concentrations of soil Olsen P and soil-solution NO_3^- and the total
392 soil N:Olsen P and soil solution NO_3^- :Olsen P ratios were more related to bulk N

393 deposition than to MAP and MAT (Tables S6 and S7). Finally, soil Olsen P and soil
394 solution P concentrations were negatively correlated ($R = - 0.37$, $P = 0.03$).

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396 RELATIONSHIP BETWEEN GROWTH AND MAP, MAT, N DEPOSITION, AND 397 NEEDLE COMPOSITION

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399 Atmospheric bulk and throughfall N deposition had a positive relationship ($R = 0.33$, P
400 < 0.0001 , and $R = 0.42$ and $P < 0.0001$, respectively) (Fig 5a and S10) with annual
401 absolute basal diameter (BD) growth over the period 1995-2005, but not with relative
402 annual BD growth ($R = 0.06$, $P = 0.38$) (data not shown). MAT was positively
403 associated with absolute annual BD growth ($R = 0.36$, $P = 0.0001$) (Fig 5b) over the
404 period 1995-2005 but not with relative annual BD growth (data not shown). There were
405 no significant relationships between MAP and absolute or relative annual BD growth
406 over the period 1995-2005 (data not shown).

407 Needle nutrient concentrations of N and P were positively associated with
408 absolute annual BD growth, together explaining 23.5% of the total variance of absolute
409 annual BD growth (Figure S11), whereas only needle P, but not N, concentration was
410 positively associated with relative annual BD growth (Figure S11). Needle potassium
411 concentration was also positively associated with absolute annual BD growth over the
412 period 1995-2005 (Figure S12), but not with relative annual BD growth. Studying the
413 relationships between “overall needle elemental composition” and growth, the PC1 axis
414 scores (explaining a 33.6% of the total variability) of the PCA analysis using needle N,
415 P, K, Ca and Mg concentrations were significantly associated with absolute annual BD
416 growth ($R=0.57$, $P<0.0001$) (data no shown) and with relative annual BD growth
417 ($R=0.30$, $P=0.001$) (data not shown). This analysis showed that needle N, P and K
418 concentrations were positively correlated with growth whereas Ca and Mg tended to be
419 negatively correlated. These results thus confirm the trends observed in the univariate
420 relationships.

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422 EFFECTS OF SOIL AND TEMPORAL TRENDS

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

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

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447 **Discussion**

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

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452 Among the environmental variables studied, MAP and N deposition were the variables
453 most strongly related to needle N concentrations. The highest needle N concentrations,
454 at 45-55°N, occurred in highly industrialized countries, such as the United Kingdom, the
455 Netherlands, Germany and Poland, in line with the highest rates of N deposition.
456 Increasing N deposition generally stimulates plant growth and net primary production
457 (Bedison & McNeil 2009; Krause *et al.* 2012), a pattern that also holds globally
458 (Fernández-Martínez *et al.* 2014). Consistent with this, in Europe we have observed a
459 positive relationship between N deposition and absolute but not relative growth of *P.*
460 *sylvestris*. We have also observed a nutrient imbalance in needles and soils due to N
461 deposition in the same period. N deposition can exceed the requirements of forest
462 growth and lead to destabilization and forest decline (Sutton *et al.* 2011; Magill *et al.*
463 2004). In general, long-term studies show an initial enhancement of tree growth that
464 with time can be followed by a tree decline linked to soil nutrient imbalances, increased
465 cation leaching from soil and changes in soil pH (Högberg *et al.* 2006; Jukyns *et al.*
466 2013). Atmospheric N deposition could thus have a positive fertilizing effect on the
467 growth of *P. sylvestris*, but this effect could be counteracted in a future when other
468 resources, such as P, become limiting. Most European areas of this study have a
469 previous long-term period of atmospheric N deposition (Sutton *et al.* 2011) which can
470 explain the observed lower needle P concentrations and higher N:P ratios in forests
471 with higher levels of N deposition.

472 However, no general temporal trend in needle N and P concentrations was
473 observed in the period 1994-2007 (Fig. 6). In this period, whereas absolute growth was
474 positively associated with both needle N and P concentrations, relative growth was only

475 related to needle P concentration. The data thus suggest that P is as much or even
476 more limiting than N across Europe. In this regard, increased growth and size of
477 individual *P. sylvestris* trees has been associated with higher needle P concentrations
478 and lower N:P ratios (Portsmouth *et al.* 2005). The lower P concentrations and higher
479 N:P ratios could hinder the growth and regeneration of *P. sylvestris*, a moderately fast-
480 growing species. In younger *P. sylvestris* stands, with higher relative growth rates, the
481 observed negative relationship between N deposition and needle P concentration,
482 especially at medium and high latitudes, could become greater. This observed higher
483 association between P (rather than N) and relative growth rates that is consistent with
484 the growth rate hypothesis (Sterner & Elser 2002), which predicts a pivotal capacitating
485 role of P in protein synthesis to sustain intense growth rates. The relationships between
486 foliar N concentrations and protein synthesis rates can, however, be complex and not
487 necessarily linear (Matzek & Vitousek 2009). These differences in needle P
488 concentrations and N:P ratios were especially large where high levels of N deposition
489 coincided with both high MAT and MAP. Hence, larger nutrient imbalances due to N
490 deposition can be expected at sites with higher productivity, where P is depleted faster.
491 The optimum MAT and MAP conditions for *P. sylvestris* are most likely met in central
492 Europe, coinciding with the centre of the species' distribution and also with the higher
493 levels of N deposition.

494 All these results further suggest that P could become increasingly limiting in
495 areas with elevated N deposition. These results are in agreement with previous
496 studies, suggesting an increase in P limitation in several forested areas at central
497 latitudes in Europe characterized by high N deposition (Harrison *et al.* 1999). An
498 increase in N deposition can stimulate soil P immobilization in forested areas by the
499 enhanced uptake of P by the soil food web (Piatek *et al.* 2009); several studies have
500 observed increased P limitation with high and continuous N loading (Phoenix *et al.*
501 2003; Gradowski & Thomas 2008). Various plant species can respond to increases in
502 N supply, improving P absorption, enhancing P uptake, or increasing the capacity to

503 reabsorb P (Fujita *et al.* 2010; Phoenix *et al.* 2004). When N loading surges, plants and
504 soil organisms enhance their mechanisms to increase P uptake, such as a higher
505 synthesis of phosphatases, but these mechanisms do not prevent an increase in the
506 N:P ratio in the long term (Peñuelas *et al.* 2013). These results also suggest that
507 climatic gradients or atmospheric N deposition can have an asymmetric impact on the
508 N and P status of plants, with a larger impact on the N:P ratio than on the individual N
509 and P status. Despite the significant role of N deposition and the higher needle N
510 concentrations in central Europe, we have also observed a trend of lower N:P ratios at
511 high latitudes relative to medium and low latitudes, suggesting a limiting role of N in
512 northern *P. sylvestris* populations (Oleksyn *et al.* 2003). In fact, these results are
513 consistent with the soil age hypothesis which claims that soils in northern ecosystems
514 are younger because the glaciers retreated only recently, and the young soils that then
515 developed are richer in P and poorer in N compared to older soils at lower latitudes.
516 Soils generally tend to evolve from N limitation to N productivity to P limitation over time
517 (Walker & Syers 1976; Chadwick *et al.* 1999). This trend was also observed in this
518 study, as *P. sylvestris* forests at lower latitudes, such as those on the Iberian and
519 Balkan Peninsulas, tended to have the highest N:P ratios, whereas the opposite was
520 observed in Scandinavian *P. sylvestris* forests at high latitudes. Despite the
521 considerable levels of atmospheric P deposition from the Saharan and Turkish deserts
522 (Mahowald *et al.* 2008; Camarero & Catalan 2012), this phenomenon did not increase
523 the needle P concentrations of *P. sylvestris* forests in either of these two peninsulas.
524 However, the low values of needle P concentrations observed in the lower latitude area
525 of distribution of *P. sylvestris* were very probably related to *P. sylvestris* forest decay
526 under the present scenario of increased drought frequency and intensity in the
527 Mediterranean area (Sanchez-Salguero *et al.* 2012; Poyatos *et al.* 2013), where strong
528 positive relationships between needle nutrient concentrations and MAP have been also
529 observed (Sardans *et al.* 2011).

530 The average N:P ratio across the 2245 *P. sylvestris* stands included in our
531 study was 10.8 ± 0.06 . Other studies of European forests have reported higher N:P
532 ratios, even in coniferous forests. For example, Sardans *et al.* (2011) reported an
533 average N:P ratio of 12.7 ± 0.4 among more than 1000 conifer stands in Catalonia
534 (Sardans *et al.* 2011). In the sites with values of N deposition of $2.5 \text{ kg N ha}^{-1} \text{ y}^{-1}$ the
535 needle N:P ratio was 8.7 on average, whereas in sites with N deposition of $20 \text{ N ha}^{-1} \text{ y}^{-1}$
536 the needle N:P ratio was 12 on average, showing that despite a certain level of leaf N:P
537 plasticity the values of N:P tend to be low N:P ratios. Low N:P ratios in photosynthetic
538 tissues are often associated with high growth rates (Sterner & Elser 2002; Elser *et al.*
539 2003; Peñuelas & Sardans 2009; Rivas-Ubach *et al.* 2012). When studying several
540 different groups of plants together, leaf N:P ratios range from 5-65 with a mean of 28-
541 30 (Elser *et al.* 2000; Elser *et al.* 2010), and thus the observed average N:P ratio of
542 10.8 ± 0.06 is in agreement with *P. sylvestris* being a relatively fast-growing species
543 occupying an early stage of forest succession (Picon-Cochard, Coll & Balandier 2006;
544 Marozas, Racinkas & Bartkevicius 2007).

545 Continuous loadings of N in *P. sylvestris* forests due to N deposition were
546 associated with an increase in the N:P ratio as a result of increasing N concentrations,
547 but also due to decreasing P concentrations. The percentage of variance of N and P
548 concentrations explained by N deposition, although significant, was low, and several
549 other factors, such as soil type and characteristics could explain a large part of the
550 variance found in the statistical analyses. Overall, variance of N and P needle
551 concentrations was particularly high in the analyses using the larger network of sites
552 when compared with using only data from the ICP Forests database. This is not
553 surprising since although the analytical techniques used were comparable in all data
554 sources, the ICP Forest database's sampling and analytical processes have
555 standardized protocols which are distinct to those used in the Spanish forest inventory
556 or to methods used to collect other data coming from very different studies. Although
557 this variance was greatest in analyses using the larger network of sites, it was still

558 relatively high when using only the ICP Forests dataset. The results strongly suggest
559 that one of the causes underlying this high variance (not explained by climate and
560 atmospheric N deposition) was soil variability. There were positive relationships
561 between soil solution nitrate and sulphate and needle N and S concentrations ($R=0.73$,
562 $P<0.0001$ and $R=0.71$, $P<0.0001$, respectively). Unfortunately, set of plots with
563 available soil type information was too small to allow a deeper analysis of this matter.
564 All in all, the negative relationships between N throughfall and bulk deposition with
565 needle P concentration would likely have a negative impact on growth of this fast-
566 growing tree species. These results suggest that N deposition could be particularly
567 negative for fast-growing species and that the N:P ratio can be a possible indicator of
568 the effects of atmospheric N deposition on plant growth or vitality, although more
569 research is needed to confirm its use.

570

571 RELATIONSHIPS BETWEEN MAP, MAT, AND ATMOSPHERIC N DEPOSITION AND
572 SOIL CHEMISTRY: ARE THEY RELATED TO SHIFTS IN NEEDLE N, P, AND S
573 CONCENTRATIONS AND N:P RATIO?

574

575 This study shows that N deposition leads to nutrient imbalances in European *P.*
576 *sylvestris* ecosystems, especially in the soils. The positive relationships of N deposition
577 with needle N concentration and of N:P ratio with soil-solution NO_3^- concentration
578 strongly suggest that European *P. sylvestris* forests have been a large sink of N but
579 tend to be N saturated in areas with higher N deposition. This is in line with most
580 studies of natural ecosystems under high levels of long-term N deposition with N
581 saturation of the soils (Aber *et al.* 2003; Pilkington *et al.* 2005). The high concentrations
582 of NO_3^- in the soil solution may suggest that *P. sylvestris* forests under higher rates of
583 N deposition are unable to immobilize all of the deposited N, thus favouring N losses by
584 leaching and/or surface runoff. These results are in agreement with previous studies
585 observing increases in soil-solution NO_3^- concentration and in N runoff and leaching in

586 temperate forests under high levels of N deposition, even when gaseous N losses
587 during nitrification and denitrification are frequently enhanced (Stuanes & Kjonaas
588 1998; Pilkington *et al.* 2005; Fatemi *et al.* 2012).

589 Our study further revealed that N deposition was negatively correlated with the
590 concentration of extractable soil Olsen P, representing P available to plants, and
591 positively with the soil solution NO_3^- :P ratio. Our results also showed that N deposition
592 was positively correlated with soil- solution P concentration. Olsen P concentration was
593 therefore negatively correlated with soil solution P concentration, thus suggesting a
594 large displacement of exchangeable P (fixed in labile bonds within the soil complex)
595 towards the soil solution. While elevated N deposition increased soil solution P
596 concentrations, deposition increased soil-solution NO_3^- concentrations even more
597 greatly, thus increasing the soil-solution NO_3^- :P ratio. As a consequence of N
598 deposition, the reserve of soil P available to plants thus gives the impression of
599 depletion, while N and P loadings to runoff increase: this may account for the high
600 needle N:P ratio in *P. sylvestris* and the high soil and soil-solution N:P ratios observed
601 under high levels of atmospheric N deposition. These high ratios under high levels of
602 atmospheric N deposition thus show that *P. sylvestris* trees are unable to maintain their
603 needle N:P ratios and P concentrations, despite the fact that the available data would
604 suggest enhanced P uptake from soils.

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

612 Positive relationships have been observed among the soil-solution
613 concentrations of K^+ , Ca^{2+} , SO_4^{2-} , and NO_3^- and N deposition. Increased concentrations

614 of Ca^{2+} in soils have also been observed in other studies and are associated with soil
615 acidification under high N and S deposition, whereas protons are exchanged with Ca^{2+}
616 or neutralized by weathering processes (Rustad *et al.* 1993; Verstraeten *et al.* 2012).
617 These observed increases in Ca^{2+} , NO_3^- , K^+ , and SO_4^{2-} concentrations suggest that
618 losses of these nutrients in runoff might increase in a manner which is proportional to
619 the level of N deposition. The increase in soil solution K^+ concentration in stands with
620 high N loadings also suggests shifts in the K cycle and N:K ratios. Although shifts in K
621 cycling and the stoichiometric relationships of K with N and P in terrestrial ecosystems
622 across natural gradients or in response to global change have been studied very little, it
623 is known that they can change across climatic gradients (Sardans *et al.* 2012c) and
624 especially in plants subjected to drought (Rivas-Ubach *et al.* 2012).

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

636

637 SCALING RELATIONSHIP BETWEEN NEEDLE N AND P CONCENTRATIONS

638

639 In this study needle N concentration scaled at 0.498 relative to P concentration (Log [N]
640 $= 0.556 + 0.498 \text{ Log [P]}$). This result is consistent with previous studies showing that
641 foliar N:P ratio decreases when foliar P (and also N) concentration increases (Niklas *et*

642 *al.* 2005; Reich *et al.* 2010), and is also consistent with what is expected according to
643 the Growth Rate Hypothesis (GRH) (Sterner & Elser, 2002). .

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

650

651 TEMPORAL TRENDS

652

653 Our results also suggest that needle S concentrations in European *P. sylvestris* forests
654 have decreased over the last two decades, while needle N and P concentrations have
655 remained more or less stable. These findings are consistent with the general patterns
656 of change in atmospheric N and S deposition in Europe over the past decades: a
657 modest reduction of N emissions associated with agriculture (Sutton *et al.* 2011) and a
658 dramatic reduction of S emissions since the early 1980s following a scaling down of
659 coal combustion and the implementation of policies to improve air quality (Eurostat
660 2009). Moreover, the efficiency of environmental policies for reducing N emissions
661 varies among European regions, with some countries still increasing emissions in
662 recent decades.

663

664 **Acknowledgements**

665

666 This research was supported by the European Research Council Synergy grant ERC-2013-SyG
667 610028 IMBALANCE-P and the European FP7-ENV ECLAIRE project, Spanish Government
668 grant CGL2013-48074-P and the Catalan Government project SGR 2014-274. Marcos
669 Fernández-Martínez was funded by the FI grant 2013 of the Catalan Government. IAJ
670 acknowledges the support of the Flemish Fund for Scientific Research. We would like to thank

671 Isaura Rábago and Marta García Vivanco from CIEMAT for helping to prepare the nitrogen-
672 deposition data. The authors acknowledge Anna Carlin Benedictow of the Norwegian
673 Meteorological Institute for providing the EMEP. The evaluations were carried out with data
674 submitted under the joint EU/ICP Forests monitoring programme (see www.icp-forests.org).
675 Specifically, data from France, Germany, United Kingdom, Spain, Sweden, Austria, Finland,
676 Spain, Switzerland, Norway, Lithuania, Estonia, Belgium, Netherlands were used. The authors
677 are also deeply indebted to the data providers and the managers of the ICP Forests data.
678

679 **Data accessibility**

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

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699 **References**

- 700
701 Aber, J.D., Goodale, C.L., Ollinger, S.V., Smith, M.L., Magill, A.H., Martin, M.E., Hallet, R.A. &
702 Stoddard, J.L. (2003) Is nitrogen deposition altering the nitrogen status of northeastern
703 forests? *Bioscience*, **53**, 375-389.
- 704 Aherne, J. & Posch, M. (2013) Impacts of nitrogen and sulphur deposition on forest ecosystem
705 services in Canada. *Current Opinion in Environmental Sustainability*, **5**, 108-1215.
- 706 Bedison, J.E. & McNeil, N.E. (2009) Is the growth of temperate forest trees enhanced along an
707 ambient nitrogen deposition gradient? *Ecology*, **90**, 1736-1742.
- 708 Boersma, M. & Elser, J.J. (2006) Too much of a good thing: on stoichiometrically balanced diets
709 and maximal growth. *Ecology*, **87**, 1325-1330.
- 710 Bragazza, L., Tahvanainen, T., Kutnar, L. *et al.* (2004) Nutritional constraints in ombrotrophic
711 *Sphagnum* plants under increasing atmospheric nitrogen deposition in Europe. *New*
712 *Phytologist*, **163**, 609-616.
- 713 Britton, A.J., Helliwell, R.C., Fisher, J.M. & Gibbs, S. (2008) Interactive effects of nitrogen
714 deposition and fire on plant and soil chemistry in an alpine heathland. *Environmental*
715 *Pollution*, **156**, 409-416.
- 716 Carnicer, J., Sardans, J., Stefanescu, C., Ubach, A., Bartrons, M., Asensio, D. & Peñuelas, J.
717 (2014) Global biodiversity, stoichiometry and ecosystem function responses to human-
718 induced C-N-P imbalances. *Journal of Plant Physiology*, In Press.
- 719 Camarero, L. & Catalan, J. (2012) Atmospheric phosphorus deposition may cause lakes to
720 revert from phosphorus limitation back to nitrogen limitation *Nature Communications*, **3**,
721 1118.
- 722 Chadwick, O.A., Derry, L.A., Vitousek, P.M., Huebert, B.J. & Hedin, L.O. (1999) Changing
723 sources of nutrients during four million years of ecosystem development. *Nature*, **397**,
724 491-497.
- 725 Cheddadi, R., Vendramin, G.G., Litt, T. *et al.* (2006) Imprints of glacial refugia in the modern
726 genetic diversity of *Pinus sylvestris*. *Global Ecology and Biogeography*, **15**, 271-282.
- 727 Core R Team (2013). R: A language and environment for statistical computing. R Foundation
728 for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- 729 Davison AC, Hinkley DV, Schechtman E (1986) Efficient Bootstrap Simulation. *Biometrika*, **73**,
730 555-566.
- 731 Elser, J.J., Fagan, W.F., Denno, R.F. *et al.* (2000) Nutritional constraints in terrestrial and
732 freshwater food webs. *Nature*, **408**, 578-580
- 733 Elser, J.J., Acharya, K., Kyle, M. *et al.* (2003) Growth rate–stoichiometry couplings in diverse
734 biota. *Ecology Letters*, **6**, 936-943
- 735 Elser, J.J., Fagan, W.F., Kerkhoff, A.J., Swenson, N.G. & Enquist, B.J. (2010) Biological
736 stoichiometry of plant production: metabolism, scaling and ecological response to global
737 change. *New Phytologist*, **186**, 593-608.
- 738 Elser, J.J., Dobberfuhl, D.R., MacKay, N.A. & Schampel J.H. (1996) Organism size, life history,
739 and N: P stoichiometry. *Bioscience*, **46**, 674-684.
- 740 EMEP (2011) Transboundary Acidification, Eutrophication and Ground Level Ozone in Europe
741 in 2009, EMEP Status Report 1/2011, Joint MSC-W & CCC & CEIP Report, ISSN 1504-
742 6109.
- 743 Eurostat (2009) Statistical books. Panorama of Energy. Energy statistics to support EU policies
744 and solutions. Edited by: Office for Official Publications of the European Communities,
745 Luxembourg.
- 746 Fatemi, F.R., Fernandez, I.J., Szillery, J., Norton, S.A. & Rustad, L.E. (2012) Soil solution
747 chemical response to two decades of experimental acidification at the bear brook
748 watershed in Maine. *Water, Air and Soil Pollution*, **223**, 6171-6186.
- 749 Fernández-Martínez, M., Vicca, S., Janssens, I.A., Luysaert, S., Campioli, M., Sardans, J.,
750 Estiarte, M. & Peñuelas, J. (2013) Spatial variability and controls over biomass stocks,
751 carbon fluxes and resource-use efficiencies across forest ecosystems. *Tress*, **28**, 597-
752 611.
- 753 Fujita, Y., de Ruiter, P.C., Wassen, M.J. & Heil, G.W. (2010) Time-dependent, species-specific
754 effects of N:P stoichiometry on grassland plant growth. *Plant and Soil*, **334**, 99-112.
- 755 García, L.V. (2004) Escaping the Bonferroni iron claw in ecological studies. *Oikos* 105: 657-663.
- 756 Gracia, C., Burriel, J.A., Ibáñez, J.J., Mata, T. & Vayreda, J. (2004) *Inventari Ecològic i Forestal*
757 *de Catalunya. Mètodes*. Volum 9. CREA, Bellaterra, 112 pp.

- 758 Gradowski, T. & Thomas, S.C. (2008) Responses of *Acer saccharum* canopy trees and saplings
759 to P, K and lime additions under high N deposition. *Tree Physiology*, **28**, 173-185.
- 760 Güsewell, S. (2004) N: P ratios in terrestrial plants: variation and functional significance. *New*
761 *Phytologist*, **164**, 243-266
- 762 Güsewell, S., Bailey, K.M., Roem, W.J. & Bedford, B.L. (2005) Nutrient limitation and botanical
763 diversity in wetlands: can fertilisation raise species richness? *Oikos*, **109**, 71-80
- 764 Harrison, A.F., Carreira, J., Poskitt, J.M. *et al.* (1999) Impacts of pollutant inputs on forest
765 canopy condition in the UK: possible role of P limitations. *Forestry*, **72**, 367-377.
- 766 Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005). Very high resolution
767 interpolated climate surfaces for global land areas. *International Journal of Climatology*,
768 **25**, 1965–1978.
- 769 Högberg, P., Fan, H., Quist, M., Binkley, D. & Tamm, C.O., (2006) Tree growth and soil
770 acidification in response to 30 years of experimental nitrogen loading on boreal forest.
771 *Global Change Biology*, **12**, 489-499.
- 772 Hughes, R.F. & Denslow, J.S. (2005) Invasion by a N-2-fixing tree alters function and structure
773 in wet lowland forests of Hawaii. *Ecological Applications*, **15**, 1615-1628.
- 774 Juknys, R., Augustaitis, A., Vencloviene, J., Kliucius, A., Vitas, A., Bartkevicius, E. & Jurkonis,
775 N. (2013) Dynamic response of tree growth to changing environmental pollution.
776 *European Journal of Forest Research*, In press.
- 777 Kattge, J., Diaz, S., Lavorel, S. *et al.* (2011) TRY - a global database of plant traits. *Global*
778 *Change Biology*, **17**, 2905-2935.
- 779 Khang, H., Zhuang, L.W., Wu, L. *et al.* (2011) Variation in leaf nitrogen and phosphorus
780 stoichiometry in *Picea abies* across Europe: an analysis based on local observations.
781 *Forest Ecology and Management*, **261**, 195-202.
- 782 Komsta, L. (2012). mblm: Median-Based Linear Models. R package version
783 0.11. <http://CRAN.R-project.org/package=mblm>
- 784 Krause, K., Cherubini, P., Bugmann, H. & Schleppei, P. (2012) Growth enhancement of *Picea*
785 *abies* trees under long-term, low-dose N addition is due to morphological more than to
786 physiological changes. *Tree Physiology*, **32**, 1471-1481.
- 787 Magill, A.H., Aber, J.D., Currie, W.S., Nadelhoffer, K.J., Martin, M.E., McDowell, W.H., Melillo,
788 J.M. & Steudler, P. (2004) Ecosystem response to 15 years of chronic nitrogen additions
789 at the Harvard Forest LTER, Massachusetts, USA. *Forest Ecology and Management*,
790 **196**, 7–28.
- 791 Mahowald, N., Jickells, T.D., Baker, A.R., Artaxo, P., Benitez-Nelson, C.R., Bergametti, G.,
792 Bond, T.C., Chen, Y., Cohen, D.D., Herut, B., Kubilay, N., Losno, R., Luo, C., Maenhaut,
793 W., McGee, K.A., Okin, G.S., Siefert, R.L. & Tsukuda, S. (2008) The global distribution of
794 atmospheric phosphorus deposition and anthropogenic impacts. *Global Biogeochemical*
795 *Cycles*, **22**, GB4026,
- 796 Marozas, V., Racinkas, J. & Bartkevicius, E. (2007) Dynamics of ground vegetation after
797 surface fires in hemiboreal *Pinus sylvestris* forests. *Forest Ecology and Management*,
798 **250**, 47-55.
- 799 Marques, R., Ranger, J., Gelhaye, D., Pollier, B., Ponette, Q. & Goedert, O. (1996). Comparison
800 of chemical composition of soil solutions collected by zero-tension plate lysimeters with
801 those from ceramic-cup lysimeters in a forest soil. *European Journal of Soil Science*, **47**,
802 407-417.
- 803 Matzek, V., Vitousek, P.M. (2009) N : P stoichiometry and protein : RNA ratios in vascular
804 plants: an evaluation of the growth-rate hypothesis. *Ecology Letters*, **12**, 765-771.
- 805 Matzek, V., Vitousek, P.M. (2009) N : P stoichiometry and protein : RNA ratios in vascular
806 plants: an evaluation of the growth-rate hypothesis. *Ecology Letters*, **12**, 765-771.
- 807 McGroddy, M.E., Daufresne, T. & Hedin, L.O. (2004) Scaling of C: N: P stoichiometry in forests
808 worldwide: implications of terrestrial Redfield-type ratios. *Ecology*, **85**, 2390-2401.
- 809 Mitchell-Olds, T. (1986) Jackknife, bootstrap and other resampling methods in regression
810 analysis. *The Annals of Statistics*, **14**, 1316-1318.
- 811 Niklas, K.J, Owens, T., Reich, P.B. & Cobb, E.D. (2005) Nitrogen/phosphorus leaf stoichiometry
812 and the scaling of plant growth. *Ecology Letters*, **8**, 636-642.
- 813 Oleksyn, J., Reich, P.B., Zytowski, R., Karolewski, P. & Tjoelker, M.G. (2003) Nutrient
814 conservation increases with latitude of origin in European *Pinus sylvestris* populations.
815 *Oecologia*, **136**, 220-235.
- 816 Peñuelas, J. & Sardans, J. (2009) Elementary factors. *Nature*, **460**, 803-804.

- 817 Peñuelas, J., Sardans, J., Rivas-Ubach, A. & Janssens, I.A. (2012) The human induced
818 imbalance between C, N and P in Earth's life-system. *Global Change Biology*, **189**, 5-8.
- 819 Peñuelas, J., Poulter, B., Sardans, J. *et al.* (2013) Human-induced nitrogen-phosphorus
820 imbalances alter ecosystems across the globe. *Nature Communications*, **4**, 2934.
- 821 Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, R.J. & Lee, J.A. (2004) Simulated
822 pollutant nitrogen deposition increases P demand and enhances root-surface
823 phosphatase activities of three plant functional types in a calcareous grassland. *New
824 Phytologist*, **161**, 279-289.
- 825 Piatek, K.B., Munasinghe, P., Peterjohn, W.T., Adams, M.B. & Cumming, J.R. (2009) Oak
826 contribution to litter nutrient dynamics in an Appalachian forest receiving elevated
827 nitrogen and dolomite. *Canadian Journal of Forest Research*, **39**, 936-944.
- 828 Picon-Cochard, C., Coll, L. & Balandier, P. (2006) The role of below-ground competition during
829 early stages of secondary succession: the case of 3-year-old Scots pine (*Pinus sylvestris*
830 L.) seedlings in an abandoned grassland. *Oecologia*, **148**, 373-383.
- 831 Pilkington, M.G., Caporn, S.J.M., Carroll, J.A. *et al.* (2005) Effects of increased deposition of
832 atmospheric nitrogen on an upland moor: leaching of N species and soil solution
833 chemistry. *Environmental Pollution*, **135**, 29-40.
- 834 Pons, X. (2009) MiraMon. Geographic information System and Remote Sensing software.
835 Centre de Recerca Ecologica i Aplicacions Forestals, CREAM. Barcelona, Spain, ISBN:
836 84-931323-5-7.
- 837 Portsmouth, A., Niinemets, Ü., Truus, L. & Pense, M. (2005) Biomass allocation and growth rates
838 in *Pinus sylvestris* are interactively modified by nitrogen and phosphorus availabilities
839 and by tree size age. *Canadian Journal of Forest Science*, **35**, 2346-2359.
- 840 Poyatos, R., Aguade, D., Galiano, L., Mencuccini, M., Martinez-Vilalta, J. (2013) Drought-
841 induced defoliation and long periods of near-zero gas exchange play a key role in
842 accentuating metabolic decline of Scots pine. *New Phytologist*, **200**, 388-401.
- 843 Prietzel, J. & Stetter, U. (2010) Long-term trends of phosphorus nutrition and topsoil
844 phosphorus stocks in unfertilized and fertilized Scots pine (*Pinus sylvestris*) stands at two
845 sites in Southern Germany. *Forest Ecology and Management*, **259**, 1141-1150.
- 846 R Core Team. (2013). R: A Language and Environment for Statistical Computing. Vienna.
847 Retrieved from <http://www.r-project.org/>
- 848 Reich, P.B. & Oleksyn, J. (2004) Global patterns of plant leaf N and P in relation to temperature
849 and latitude. *Proceedings of the National Academy of Science USA*, **101**, 11001
- 850 Reich, P.B., Oleksyn, J., Wright, I.J., Niklas, K.J., Hedin, L. & Elser, J.J. (2010) Evidence of a
851 general 2/3-power law of scaling leaf nitrogen to phosphorus among major plant groups
852 and biomes. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 877.
- 853 Richardson, S.J., Allen, B. & Doherty, J.E. (2008) Shifts in leaf N:P ratio during reabsorption
854 reflect soil P in temperate forest. *Functional Ecology*, **22**, 738-745.
- 855 Rivas-Ubach, A., Sardans, J., Pérez-Trujillo, M., Estiarte, M. & Peñuelas, J. (2012) Strong
856 relationship between elemental stoichiometry and metabolome. *Proceedings of the
857 National Academy of Sciences USA*, **109**, 4181-4186.
- 858 Robroek, B.J.M., Adema, E.B., Venterink, H.O., Leonardson, L., Wassen, M.J. (2009) How
859 nitrogen and sulphur addition, and a single drought event affect root phosphatase activity
860 in *Phalaris arundinacea*. *Science of The Total Environment*, **407**, 2342-2348.
- 861 Rowe, E.C., Smart, S.M., Kennedy, V.H., Emmett, B.A. & Evans, C.D. (2008) Nitrogen
862 deposition increases the acquisition of phosphorus and potassium by heather *Calluna
863 vulgaris*. *Environmental Pollution*, **155**, 201-207.
- 864 Rustad, L.E., Fernandez, I.J., Fuller, R.D., David, M.B., Nodvin, S.C. & Halteman, W.A. (1993)
865 Soil solution response to acidic deposition in a northern hardwood forest. *Agriculture,
866 Ecosystems and Environment*, **47**, 117-134.
- 867 Sanchez-Salguero, R., Navarro-Carrillo, R.M., Camarero, J.J., Fernandez-Cancio, A. (2012)
868 Selective drought-induced decline of pine species in southeastern Spain. *Climatic
869 Change*, **113**, 767-785.
- 870 Sardans, J., Rivas-Ubach, A. & Peñuelas, J. (2011) Factors affecting nutrient concentration an
871 stoichiometry of forest trees in Catalonia (NE Spain). *Forest Ecology and Management*,
872 **262**, 2024-2034.
- 873 Sardans, J., Rivas-Ubach, A. & Peñuelas, J. (2012a) The C:N:P stoichiometry of organisms and
874 ecosystems in a changing world: a review and perspectives. *Perspectives in Plant
875 Ecology, Evolution and Systematics*, **14**, 33-47.

- 876 Sardans, J., Rivas-Ubach, A. & Peñuelas, J. (2012b) The elemental stoichiometry of aquatic
877 and terrestrial ecosystems and its relationships with organism life style and ecosystem
878 structure and function: a review. *Biogeochemistry*, **111**, 1-39.
- 879 Sardans, J., Peñuelas, J., Coll, M., Vayreda, J. & Rivas-Ubach, A. (2012c) Stoichiometry of
880 potassium is largely determined by water availability and growth in Catalanian forests.
881 *Functional Ecology*, **26**, 1077-1089.
- 882 Sardans, J. & Peñuelas, J. (2013) Tree growth changes with climate and forest type are
883 associated with relative allocation of nutrients, especially phosphorus, to leaves and
884 wood. *Global Ecology and Biogeography*, **22**, 494-507.
- 885 Seastedt, T.R. & Vaccaro, L. (2001) Plant species richness, productivity, and nitrogen and
886 phosphorus limitations across a snowpack gradient in Alpine Tundra, Colorado, USA.
887 *Arctic Antarctic and Alpine Research*, **33**, 100-106.
- 888 Sen, P.K. (1968) Estimates of the regression coefficient based on Kendall's tau. *Journal of the*
889 *American Statistical Association*, **63**, 1379-1389.
- 890 Simpson, D., Benedictow, A., Berge, H., Bergstrom, R., Emberson, L.D., Fagerli, H., Flechard,
891 C.R., *et al.* (2012). The EMEP MSC-W chemical transport model - technical description.
892 *Atmospheric Chemistry and Physics*, **12**, 7825-7865.
- 893 Sistla, S.A. & Schimel, J.P. (2012) Stoichiometry flexibility as a regulator of carbon and nutrient
894 cycling in terrestrial ecosystems under change. *New Phytologist*, **196**, 68-78.
- 895 Sterner, R.W. & Elser, J.J. (2002) Ecological Stoichiometry: The Biology of Elements from
896 Molecules to the Biosphere. Princeton University Press.
- 897 Stuanes, A.O. & Kjonaas, O.J. (1998) Soil solution during four years of NH₄ NO₃ addition to a
898 forested catchment at Gardsjön, Sweden. *Forest Ecology and Management*, **1001**, 215-
899 226.
- 900 Sutton, M.A., Howard, C.M., Erismann, J.W., Billen, G., Bleeker, A., Grennfelt, P., Grinsven, H.V.
901 & Grizzetti, B. (eds) (2011) The European Nitrogen Assessment (Cambridge Univ.
902 Press).
- 903 Sykes, M.T. & Prentice, I.C. (1995) Boreal forest futures – Modeling the controls on tree species
904 range limits and transient responses to climate change. *Water Air and Soil Pollution*, **82**,
905 415-428.
- 906 Theil, H. (1950) A rank-invariant method of linear and polynomial regression analysis.
907 *Nederlandse Akademie Wetenschappen Series A*, **53**, 386-392.
- 908 Van Dijk, J., Robroek, B., Kardel, I. & Wassen, M. (2012) Combined effects of nitrogen
909 enrichment, sulphur pollution and climate change on fen meadow vegetation N:P
910 stoichiometry and biomass. *Biogeochemistry*, **111**, 139-150.
- 911 Verstraeten, A., Neiryneck, J., Genouw, G., Cools, N., Roskams, P. & Hens, M. (2012) Impact of
912 declining atmospheric deposition on forest soil solution chemistry in Flanders, Belgium.
913 *Atmospheric Environment*, **62**, 50-63.
- 914 Villanueva, J.A. (ed). (2005) *Tercer Inventario Forestal Nacional (1997-2007)*. Ed. Ministerio de
915 Medio Ambiente. Madrid.
- 916 Vitousek, P.M., Turner, D.R. & Kitayama, K. (1995) Foliar nutrients during long-term soil
917 development in Hawaiian Montane rain-forest. *Ecology*, **76**, 712-720.
- 918 Vrede, T., Andersen, T. & Hessen, D.O. (1999) Phosphorus distribution in three crustacean
919 zooplankton species. *Limnology and Oceanography*, **44**, 225-229.
- 920 Vrede, T., Dobberfuhl, D.R., Kooijman, S. & Elser, J.J. (2004) Fundamental connections among
921 organism C : N : P stoichiometry, macromolecular composition, and growth. *Ecology*, **85**,
922 1217-1229.
- 923 Walker, T.W. & Syers, J.K. (1976) Fate of phosphorus during pedogenesis. *Geoderma*, **15**, 1-19
- 924 Willby, N.J., Pulford, I.D. & Flowers, T.H. (2001) Tissue nutrient signatures predict herbaceous-
925 wetland community responses to nutrient availability. *New Phytologist*, **152**, 463-481.
- 926 Wood, S.N. (2000). Modelling and smoothing parameter estimation with multiple quadratic
927 penalties. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, **62**,
928 413-428.
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936 Figure captions
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938 Figure 1. Needle N (a) and P (b) concentrations, and N:P ratio (c) in 2245 *P. sylvestris*
939 forests stands across Europe.

940

941 Figure 2. Latitudinal gradients of needle N (a) and P (b) concentrations and N:P ratio
942 (c) in 2245 *P. sylvestris* forests stands across Europe. Latitude in North degrees. Radj =
943 R^2 squared Pearson coefficient adjusted. Tau = Kendall's tau (correlation).

944

945 Figure 3. Relationships between needle N concentration (a) N:P ratio (b) and needle P
946 concentration in 2245 *P. sylvestris* forests stands across Europe. Tau = Kendall's tau
947 (correlation).

948 Figure 4. Relationships between needle N (a) and P (b) concentrations and N:P ratio
949 (c) with atmospheric N deposition in 2245 *P. sylvestris* forests stands across Europe. z
950 = Z-statistic.

951

952 Figure 5. Relationships between annual absolute BD growth and total annual bulk N
953 deposition (a) and MAT (b) in 189 *P. sylvestris* forests stands across Europe (ICP
954 data). R = Pearson coefficient.

955

956 Figure 6. Temporal series of the needle N (a), P (b), and S (d) concentrations in 90 *P.*
957 *sylvestris* stands across Austria, Belgium, Estonia, Finland, France, Germany,
958 Lithuania, the Netherlands, Norway, Spain, Sweden, and the United Kingdom in the
959 period 1994-2007 (ICP data).

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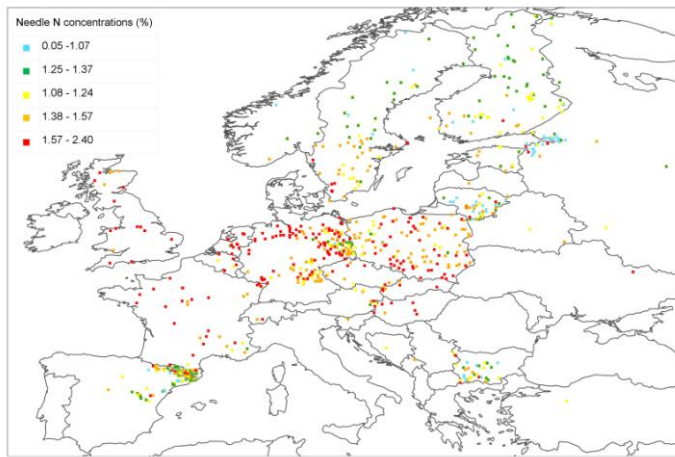
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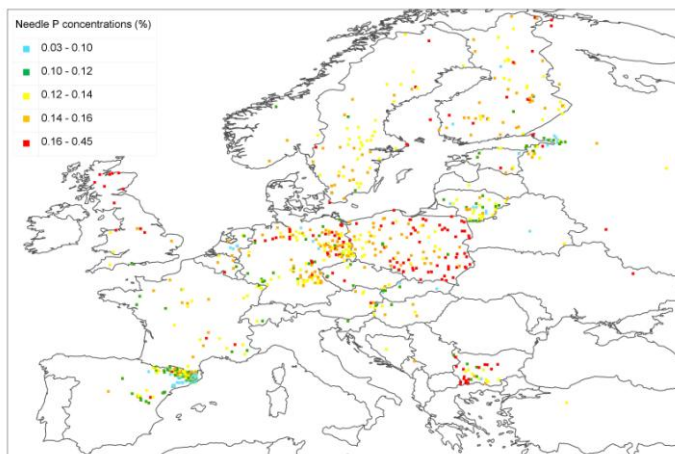
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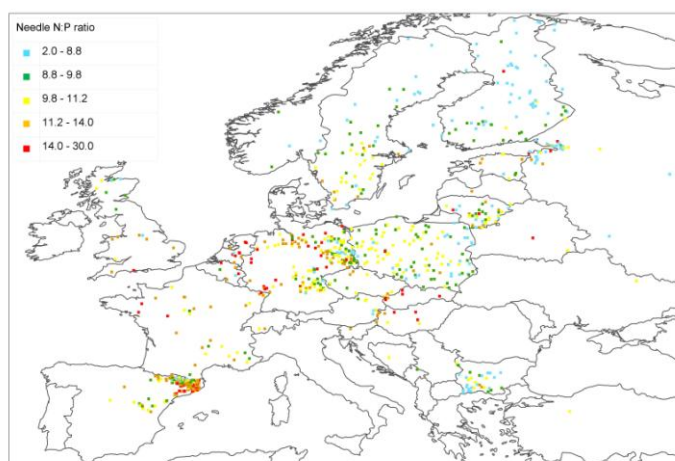
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(A)



(B)



(C)

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Figure 1

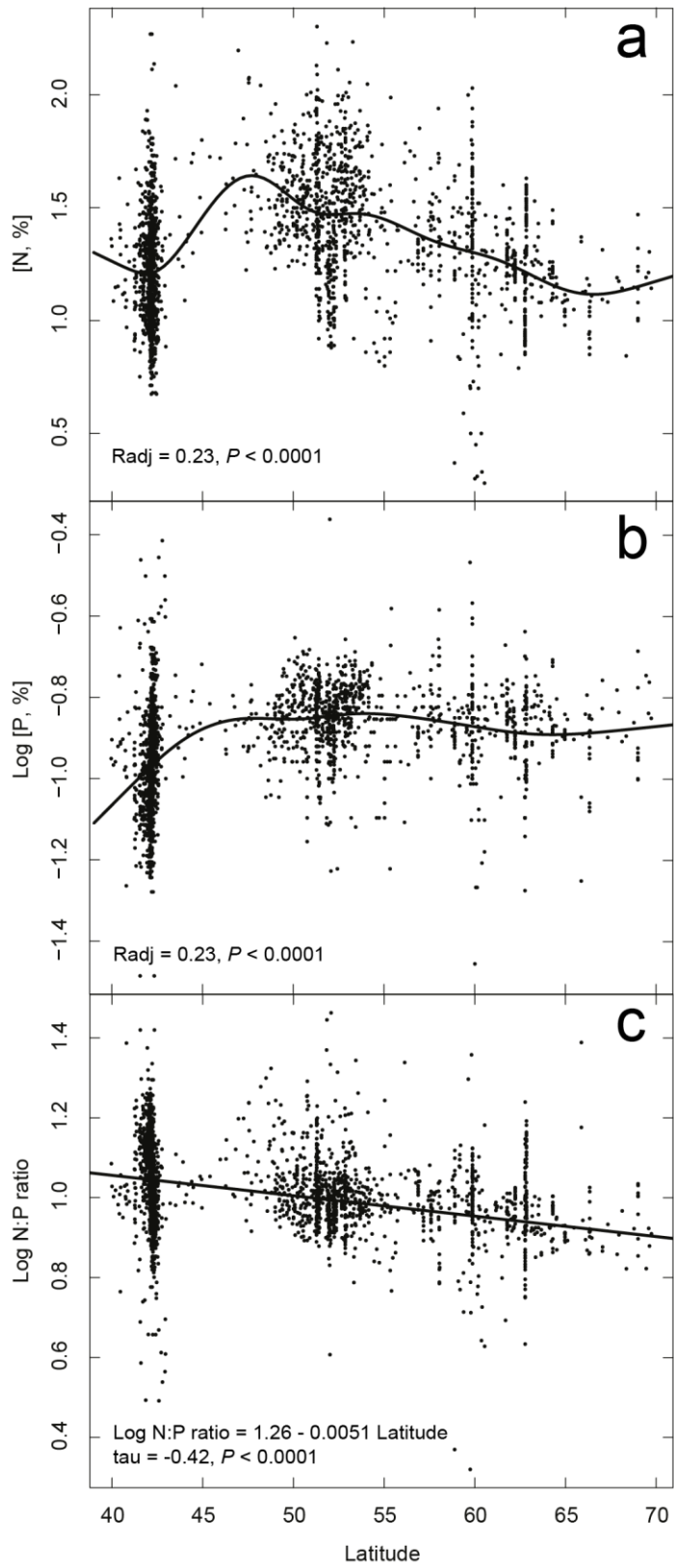


Figure 2

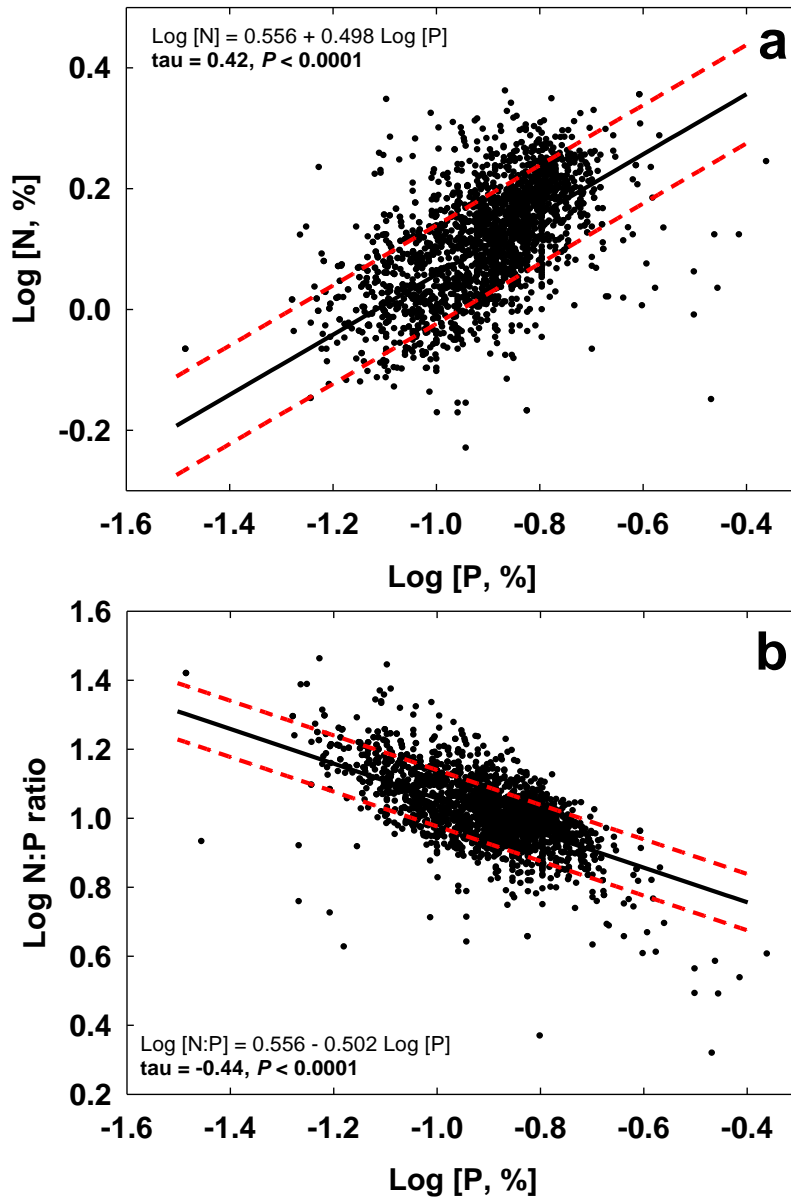


Figure 3

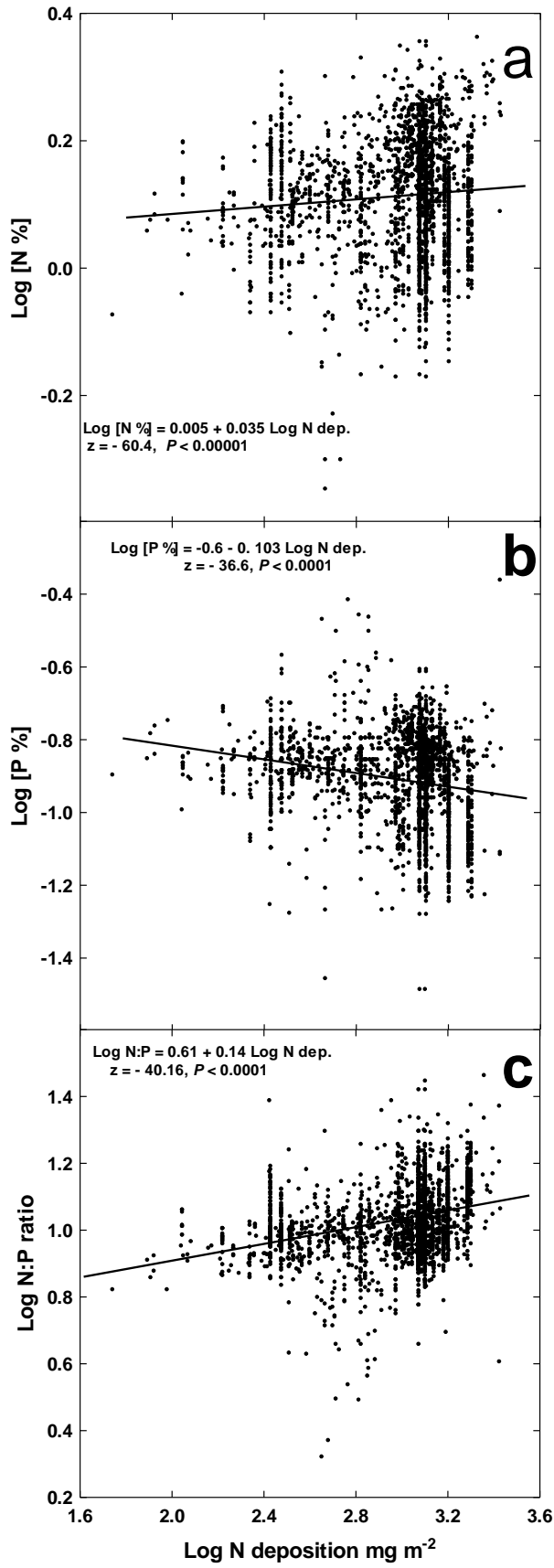


Figure 4

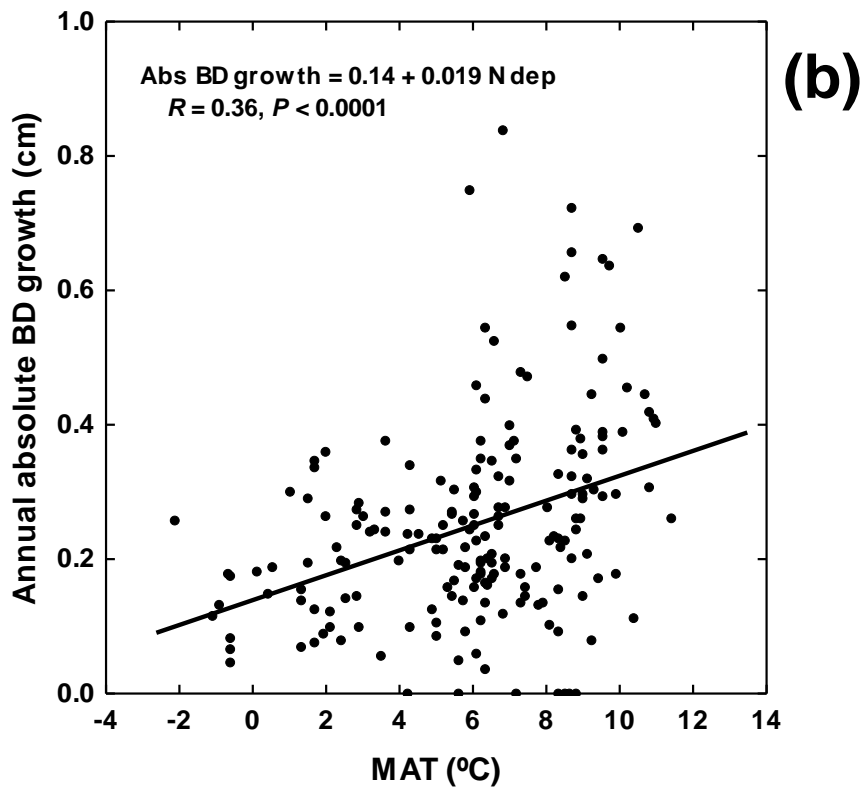
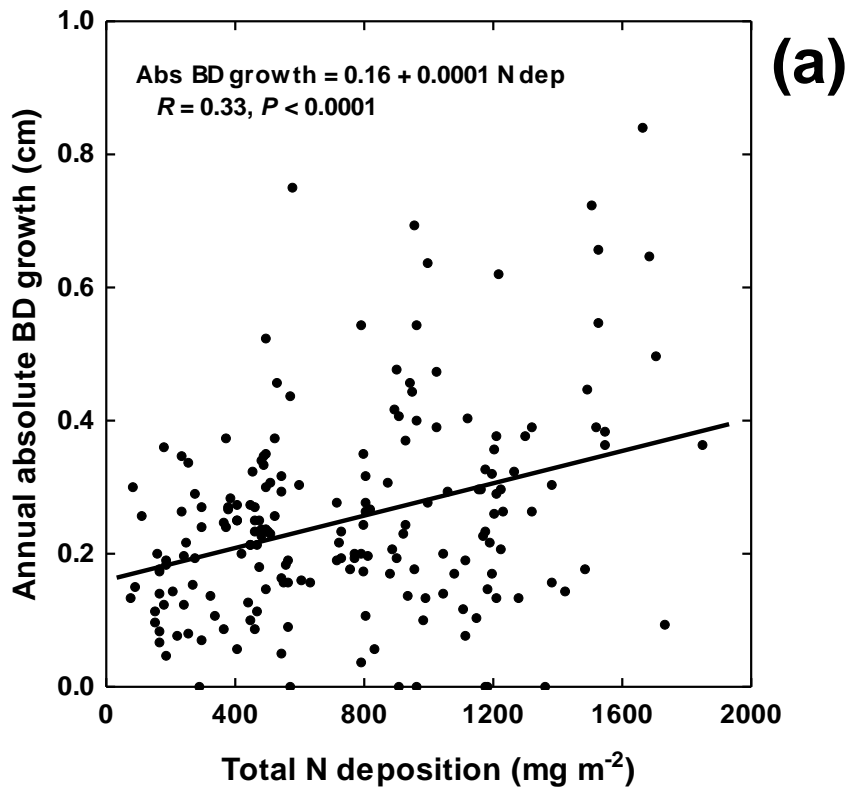


Figure 5

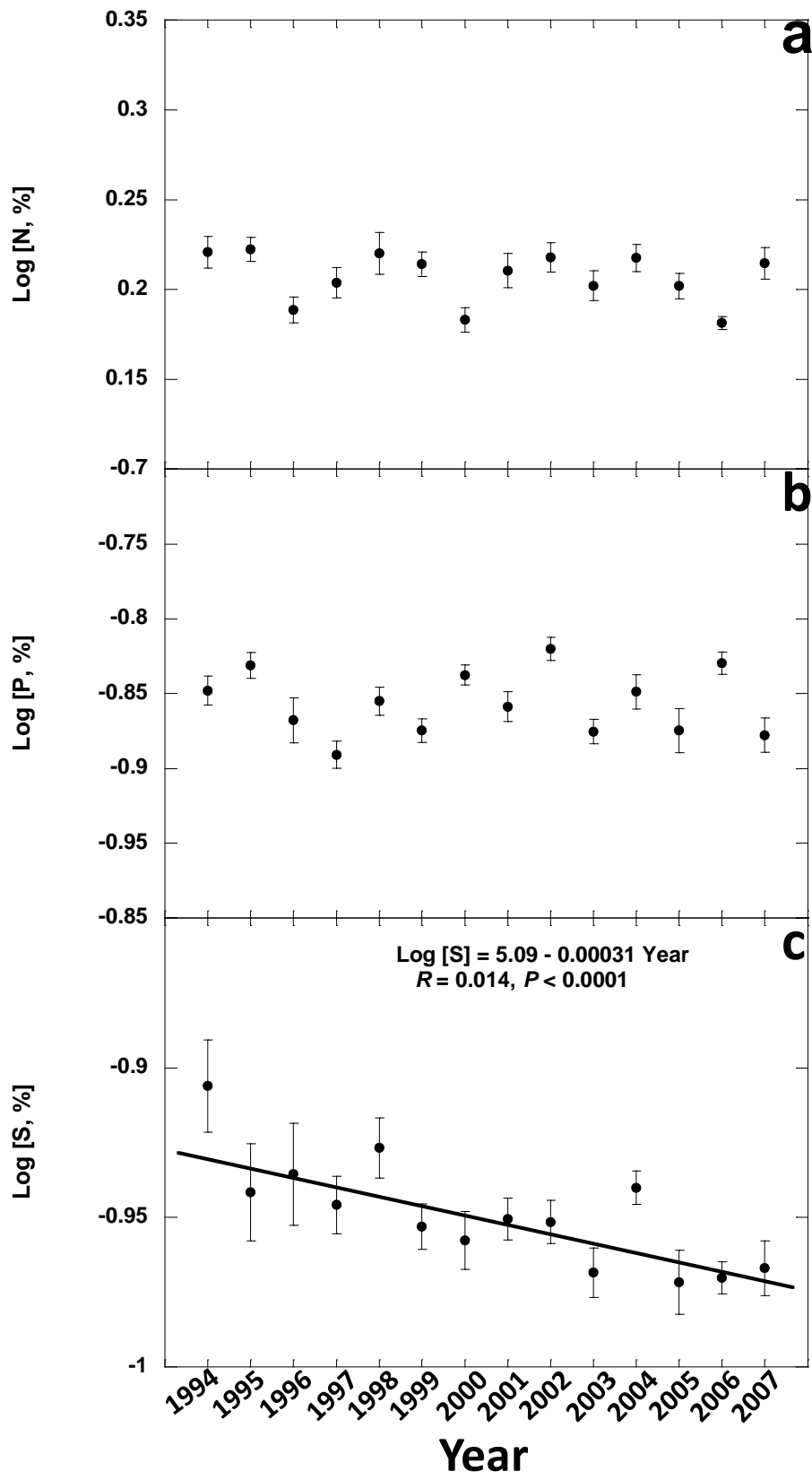


Figure 6

Table 1. Summary of the independent and dependent variables used in the different statistical analyses. The number of plots available for carrying out the analysis, the source of *P. sylvestris* data and the Tables and Figures of the manuscript and supplementary material where the results are shown are also indicated.

Independent factors	Dependent factors	Plots number	Figure and Tables where shown	Source of <i>P. sylvestris</i> data
Latitude MAP, MAT (WorldClim database) N deposition (EMEP)	Needle N and P concentration Needle N:P ratio	2245	Figures 1, 2, 3, 4 Table S1, S7 Fig S3, S4	TRY ICP Ecological Forest Inventory of Catalonia Third Spanish National Forest Inventory ISI WEB of Science
MAP, MAT (WorldClim database) N deposition (EMEP)	Needle N and P concentration Needle N:P ratio	67 (570 tree data)	Table S2 Figure S13	ICP
MAP, MAT (WorldClim database) N deposition (EMEP)	BD growth	189	Figure 5	ICP
N deposition (ICP)	BD growth	82	Figure S10	ICP
Needle N, K, Ca, Mg and P concentration	BD growth	82-119	Figure S11, S12	ICP
MAP, MAT (WorldClim database) N deposition (ICP)	Needle N, S and P concentration	52-55	Table S3 Fig S5	ICP
MAP, MAT (WorldClim database) N deposition (EMEP)	Soil solution NO ₃ ⁻ , K ⁺ , Ca ²⁺ , SO ₄ ²⁻ Soil P-Olsen	54-67	Table S4 Figure S1, S19, S14	ICP
MAP, MAT (WorldClim database) N deposition (ICP)	Soil solution NO ₃ ⁻ , K ⁺ , Ca ²⁺ , SO ₄ ²⁻ Soil P-Olsen	41-36	Table S5, Figure S15	ICP
MAP, MAT (WorldClim database) Soil solution NO ₃ ⁻ , SO ₄ ²⁻	Needle N, S and P concentration Needle N:P ratio	67	Table S8, Fig S12	ICP
Soil type	Needle N, S, K, Ca, Mg and P	30	Table S8	ICP

	concentration Needle N:P ratio Soil solution NO ₃ ⁻ , K ⁺ , Ca ²⁺ , SO ₄ ²⁻ Soil P-Olsen			
MAP, MAT (WorldClim database) N deposition (ICP)	Needle N, S and P concentration Needle N:P ratio	52-54		ICP
Temporal series 1994-2007 (ICP)	Needle N, S and P concentration	90	Figure 6	ICP
Throughfall N deposition (ICP) versus bulk deposition (EMEP)		98	Figure S7	