Research paper

Long-term temporal relationships between environmental conditions and xylem functional traits: a meta-analysis across a range of woody species along climatic and nitrogen deposition gradients

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The objectives of this study were to provide a quantitative description of the long-term effects of environmental variability on xylem functional traits, in order to better assess xylem capacity to change in response to climate change. Twenty-six sites throughout the world, primarily in Europe, were chosen where results from long-term measurements of anatomical traits were previously published. Published data on long-term xylem anatomy (conduit size and density) and ring width variability were compiled across a range of tree species, which was subsequently related to variability in temperature, precipitation and nitrogen deposition rates across the study sites using generalized additive models and Bayesian methods. We found some appreciable relationships between xylem traits (conduit area A_c and conduit density D_c) and environmental variables; whereas combined trait indices (lumen fraction: $A_c \times D_c$ and vessel composition: A_c/D_c) were found to be rather constant across a wide range of environmental conditions and to be decoupled from tree growth rates. Overall, results suggested xylem traits coordinated towards a homeostasis in xylem function, which appeared to act across a wide range of environmental conditions. Results showed also nitrogen deposition was associated with xylem traits and vessel composition: increased nitrogen availability due to nitrogen deposition might facilitate construction of a xylem structure efficient for water transport, and concurrently provide capacity to withstand the risks of drought-induced embolism.

Introduction

Under present and future climate change, plant phenotypic plasticity is now regarded as an important response that woody species use to cope with current accelerated rates of environmental change (Fonti et al. 2010). For example, forecasted changes in precipitation and temperature have intensified drought scenarios, which are expected to have detrimental effects on trees and forest communities in southern and mid latitude regions, as well as climate warming, which has the potential to affect plant phenology and vegetative growth throughout northern latitudes (IPCC 2014).

Nitrogen deposition due to anthropogenic perturbation of the nitrogen cycle is also an important component of global change (Schlesinger 2009), and through altered nitrogen cycling and soil nitrogen availability, it has the potential to affect the forest carbon cycle and trees' physiological properties (e.g. Magnani et al. 2007 and Leonardi et al. 2012 among the others). Therefore, the quantitative assessment of the role of nitrogen deposition in tuning trees' plasticity might have important implications for our understanding of how tree species will respond to global change.

Modified patterns in carbon allocation to roots, stems and leaves might represent an effective plastic response, which tunes plant hydraulic architecture toward better efficiency and safety under new environmental conditions (Magnani et al. 2002). At seasonal and annual scales modifications in xylem structure, in particular changes in conduit dimensions and density, should also be considered as important plastic responses to cope under altered climatic conditions (Bryukhanova and Fonti 2013).

Xylem is a functional adaptive structure and a sensitive ecological device, designed to conduct water from roots to leaves safely and efficiently, maintain open stomata and enable carbon uptake (Carlquist 2001). Safety and efficiency are largely influenced by xylem structure: conduit dimensions, for instance, are positively related to the water amount capable of transport through the system, as predicted by the Hagen-Poiseuille formula. Thus, wider conduits contribute to enhanced hydraulic conductance; Brodribb and Field (2000) reported a correlation with enhanced hydraulic conductance, higher leaf-level photosynthesis and faster growth in conifers and angiosperm trees. However, wider conduits are at increased risk of cavitation and drought-induced embolism, with the consequence of impaired water transport and reduced plant assimilation (Tyree and Sperry 1989). In dry habitats, plant drought tolerance was considered more important than the ability to rapidly transport water, and consequently it might be more crucial for plants to optimize xylem safety (avoidance of drought-induced embolisms) compared with optimizing xylem efficiency (high hydraulic conductance) (Nardini and Tyree 1999).

Gleason et al. (2015) recently surveyed representatives of the world's woody plant species and documented a substantial range of xylem efficiency and safety levels among species and habitats. However, a weak trade-off between xylem hydraulic efficiency and safety was found at the species level; therefore no clear evolutionary strategy to maximize efficiency at a given safety level appeared to operate (Gleason et al. 2015).

However, this finding does not preclude the hypothesis that throughout a tree's life, at different temporal scales, plastic responses in xylem structure might occur, with the result of preserving efficiency and safety. Indeed, Hacke et al. (2006) reported that xylem has the potential to acclimate its structure to compensate for cavitation risk by vessel number and distribution modifications, or by vessel clustering. In addition, a rather large number of long-term tree-ring anatomical studies have demonstrated changes in conduit dimension and density throughout an individual tree's life are sensitive indicators of ecological change, with strong effects from environmental variables (sequence of climatic events, atmospheric CO_2 concentration and nutrient availability, among others) on wood anatomical structure (see Fonti et al. 2010, for review). Moreover in several cases, studies have shown that xylem structure encoded valuable environmental information; for example, rather clear relationships between functional anatomical traits and temperature were reported for conifer species growing in temperature-limited environments (Bryukhanova and Fonti 2013, Fonti et al. 2013, Pacheco et al. 2016), and recently insight from a reciprocal transplant showed vessel diameter was highly plastic in response to different environments and varyied with summer moisture availability in a boreal tree (Schreiber et al. 2015). Climatic signals on xylem structure were found also in oak species (González and Eckstein 2003); however, less evident relationships between xylem anatomy and climatic drivers were generally shown for deciduous tree species growing in temperate environments, suggesting that tree acclimation to climatic variability might result from the coordination of several complementary traits (Gea-Izquierdo et al. 2013).

Nevertheless, quantitative assessments of the long-term effects of environmental variables on xylem functional anatomical traits across a range of woody species remain unavailable. These evaluations are required to highlight general response patterns, and in particular, to examine whether effective plastic responses are occurring, along which direction, and if the responses facilitate an efficient and safe means for trees to cope under changing environmental conditions.

In the present study, we compiled data on long-term variability in xylem anatomy from published studies on woody species belonging to different anatomical functional groups (conifer, diffuse-porous and ring-porous species), and examined the relationships between these data and variability in environmental conditions. We previously considered environmental variability in terms of long-term climatic variation (temperature and precipitation) and nitrogen deposition; however, the latter has not yet been analyzed as a potential driver of long-term variation and acclimation in xylem structure. Previous studies have shown variable effects of nitrogen availability on xylem structure and function: positive effects were found on hydraulic conductivity, with decreased wood density and cell wall thickness (Kostiainen et al. 2004, Hacke et al. 2010, Plavcová et al. 2013); more long-term manipulation in conifers showed limited nitrogen availability effects on xylem cell enlargement, wall thickening and lignification (Dao et al. 2015). Interestingly, a possible increase of both xylem efficiency and cavitation resistance with nutrient availability is depicted for tree species by Goldstein et al. (2013).

In our study, we tested therefore the following hypotheses: (i) xylem traits show a plastic response to environmental conditions and trait co-variation occurs across a tree's life, where xylem safety and efficiency could be favored; and (ii) increased nitrogen availability, due to atmospheric nitrogen deposition, might represent a novel resource trees use for improved acclimation to new climatic conditions.

Materials and methods

Data set compilation

The ISI Web of Science database was used to search the primary literature. Twenty-five studies reporting quantitative

wood anatomy data from time-series dated woody rings from 21 tree species [five conifer, seven diffuse-porous and nine ring-porous; following Schweingruber (1990) and the InsideWood project database http://insidewood.lib.ncsu.edu] growing at 26 different sites were compiled (Table 1, and also see Figure S1 and Table S1 available as Supplementary Data at *Tree Physiology* Online). We considered papers published until March 2015. Based on descriptions derived from the original publications, we selected studies where evidence of forest manipulation or disturbance was not reported. All studies were conducted in natural forest stands, with the exception of Schume et al. (2004), who reported *Populus* plantation data; in the case of manipulative studies (control versus treatment),

only control data were included. Data provided in graphical form in the original studies were digitized using Un-Scan-It 5.0 software (Silk Scientific, Orem, UT, USA). Based on measurements performed in each study, the following traits were compiled in the data set and analyzed: tree-ring width (W_r), average conduit area (A_c) and conduit density (D_c). Interpolation was not applied in any case. In some cases, average conduit area was computed from xylem conduit diameter, assuming a circular form for xylem conduits. In ring-porous species, we considered conduit area and conduit density available for early-wood ring portions. Tree age was annotated when specified in the original studies and cambial age (*Age*) using tree-ring chronologies was estimated accordingly.

Table 1. Data set characteristics. Tree species with wood type (C, conifer; Dp, diffuse porous; Rp, ring-porous); site ID and geographic coordinates (Lat, latitude; Long, longitude in decimal degrees, positive numbers indicate north and east); N_{chr}, number of tree-ring chronologies compiled in the data set from the original studies; N_{tree}, number of trees that contributed to tree-ring chronologies in the original studies; Int, time intervals covered by tree-ring chronologies; Traits, xylem anatomical traits utilized from each study (W_r, tree-ring width; A_c, average conduit area; Di_h, hydraulic diameter; D_c, conduit density); Per, period estimated more important for ring formation, numbers indicate the sequence of months in the year (e.g. 4–7 = April to July, 10–1 = October to January).

Species	ID	Lat	Long	N_{chr}	N_{tree}	Int	Traits	Per	Reference
Pinus sylvestris C	1	52.38	13.07	1	8	1900–2009	A_c, D_h	4–7	Liang et al. (2013)
Larix sibirica C	2	50.38	89.07	1	25	1900–2007	W_r	5–7	Fonti et al. (2013)
Quercus spp. Rp	3	49.10	6.55	1	37	1961–1979	A_c	5–7	Huber (1993)
Quercus macrocarpa Rp	4	49.03	−97.20	4	4	1900–2000	A_c	5–7	George et al. (2002)
Populus x euramericana Dp	5	48.39	15.85	1	3	1968–1996	A_c, D_c	4–7	Schume et al. (2004)
Quercus petraea Rp	6	47.38	8.44	1	15	1956–2006	A_c, W_r	5–7	Fonti and García-González (2008)
Quercus petraea Rp	7	46.35	8.60	1	12	1900–2002	A_c, W_r	5–7	Fonti et al. (2009)
Larix decidua C	8	46.31	7.74	1	15	1960–2010	A_c W_r	5–7	Bryukhanova and Fonti (2013)
Picea abies C	8	46.31	7.74	1	15	1960–2010	A_c ,W_r	4–7	Bryukhanova and Fonti (2013)
Quercus pubescens Rp	9	46.32	7.56	1	15	1956–2006	A_c, W_r	5–7	Fonti and García-González (2008)
Quercus pubescens Rp	9	46.32	7.56	1	21	1970–1985	A_c, W_r	5–7	Eilmann et al. (2006)
Pinus sylvestris C	9	46.32	7.56	1	21	1970–1985	A_c, W_r	4–7	Eilmann et al. (2006)
Pinus sylvestris C	10	46.27	7.43	1	15	1900–2005	W_r	4–7	Eilmann et al. (2009)
Quercus pubescens Rp	10	46.27	7.43	1	15	1900–2005	W_r	5–7	Eilmann et al. (2009)
Quercus petraea Rp	11	46.19	8.88	1	15	1956–2006	A_c, W_r	5–7	Fonti and García-González (2008)
Quercus robur Rp	12	45.90	15.42	2	6	1970–2008	A_c, D_c, W_r	5–7	Gricar et al. (2013)
Ilex aquifolium Dp	13	45.90	8.95	1	10	1925–2012	A_c, D_c	4–7	Rita et al. (2015)
Quercus alba Rp	14	45.48	−75.9	1	1	1900–1990	A_c	5–7	Tardif and Conciatori (2006)
Quercus robur Rp	15	43.40	−8.05	1	6	1925–1996	A_c, W_r	5–7	González and Eckstein (2003)
Arbutus unedo Dp	16	42.78	10.21	1	1	1967–2009	A_c, D_c, W_r	5–7	Gea-Izquierdo et al. (2013)
Erica arborea Dp	16	42.78	10.21	1	1	1968–2009	A_c, D_c, W_r	4–7	Gea-Izquierdo et al. (2013)
Quercus faginea Rp	17	42.18	−0.44	4	40	1975–2007	A_c, W_r	5–7	Alla and Camarero (2012)[a]
Juniperus thurifera C	18	41.77	-2.82	1	20	1965–2004	W_r	4–7	Olano et al. (2013)
Quercus ilex Dp	19	41.33	1.83	1	10	1984–2004	A_c,W_r	4–7	Campelo et al. (2010)
Quercus faginea Rp	20	41.12	−1.65	1	10	1970–2000	D_c, Di_h, W_r	5–7	Corcuera et al. (2004b)
Quercus ilex Dp	20	41.12	−1.65	1	10	1970–2000	Di_h, W_r	4–7	Corcuera et al. (2004a)
Quercus pyrenaica Rp	20	41.12	−1.65	1	10	1970–2000	Di_h, A_c, W_r	5–7	Corcuera et al. (2006)
Erica arborea Dp	21	40.85	−3.94	1	2	1968–2009	W_r	4–7	Gea-Izquierdo et al. (2013)
Acer pseudoplatanus Dp	22	39.93	16.32	1	9	1932–2012	A_c, D_c, Di_h, W_r	4–7	Rita (2015)
Alnus cordata Dp	22	39.93	16.32	1	9	1930–2012	A_c, D_c, Di_h, W_r	4–7	Rita (2015)
Ilex aquifolium Dp	22	39.93	16.32	1	9	1930–2012	A_c, D_c, Di_h, W_r	4–7	Rita et al. (2015)
Quercus ilex Dp	23	38.60	−7.25	1	11	1942–2001	A_c, D_c, W_r	4–7	Abrantes et al. (2013)
Quercus canariensis Rp	24	36.52	−5.57	3	53	1975–2008	A_c, Di_h, D_c	5–7	Gea-Izquierdo et al. (2012)[a]
Quercus canariensis Rp	25	36.46	8.32	2	45	1975–2008	Di_h, D_c	5–7	Gea-Izquierdo et al. (2012)[a]
Prosopis flexuosa Rp	26	−32.35	−65.97	1	21	1940–2004	W_r	10–1	Giantomasi et al. (2009)

[a]Different sites reported in these studies, with very similar latitude and longitude, were shown with average geographic coordinates in this table.

From conduit size (A_c) and density (D_c), two additional indices were computed and further analyzed for their variability and dependence under changing environmental conditions: the so-called vessel composition ($S = A_c/D_c$), which is an index of vessel characteristics within the tree ring and lumen frequency ($F = A_c \times D_c$), which represents the relative amount of tree ring covered by water-conducting vessels; these indices are considered useful indicators of vascular strategy (Carlquist 2001, Zanne et al. 2010). D_c was only available for hardwood species, therefore F and S were not computed for conifer species.

Environmental variables

For each of the 26 sites, monthly climatic variables for each year during the 1900–2005 period were extracted from the 0.5° × 0.5° CRU TS 2.1 global climatic gridded data set available for download from the Climatic Research Unit of University of East Anglia, Norwich, UK (http://www.cru.uea.ac.uk/cru/data/hrg/). From monthly values, mean annual temperature (T_m) and total annual precipitation (P) were calculated.

Information available in the original studies and open literature was employed to estimate the more critical time interval in ring formation and growth for each species/site combination (Table 1). Furthermore, average temperature (T_g) and total precipitation (P_g) values were computed for this time interval.

For each of the same sites, mean annual total atmospheric reactive nitrogen deposition (N_{dep}) was estimated for the 1960–2004 period using a TM4 chemistry-transport model simulation (Schultz et al. 2007). Total reactive N includes both oxidized and reduced reactive components. The simulation was conducted at a 3° (longitude) × 2° (latitude) horizontal resolution and the results successively interpolated to a 0.5° × 0.5° grid.

Selected sites were characterized by a large range of environmental conditions, indicated by broad mean annual temperature (T_m) gradients and a wide range of total annual precipitation (P) and nitrogen deposition (N_{dep}) values: T_m ranged between −10 and 19.2 °C, P between 217 and 3031 mm, and N_{dep} between 0.04 and 2.92 g m^{-2} year^{-1}. Large differences among sites remained when climatic variables were considered for the estimated ring formation period: the average temperature over this interval (T_p) and the total precipitation (P_p) ranged between −2.60 and 24.5 °C and between 2.1 and 1618 mm, respectively (see Figure S2 available as Supplementary Data at *Tree Physiology* Online).

Annual atmospheric CO_2 concentration (C_a) values were obtained from McCarroll and Loader (2004). According to these data, C_a showed a respective increase from 285 to 312, and 312 to 368.5 µmol mol^{-1} from 1850 to 1950, and from 1950 to 2000, respectively. We assumed trees experienced well-mixed atmospheric conditions, and a single value of C_a was applied to different sites in a given year.

Statistical methods

Tree species exhibited substantial differences in xylem traits; notable distinctions were also evident when species were assembled into functional anatomical groups (conifer, diffuse-porous and ring-porous). However, the distribution of xylem traits across environmental gradients was rather unbalanced, with the exception of nitrogen deposition (see Figure S3 available as Supplementary Data at *Tree Physiology* Online).

For this reason, all statistical analyses were performed on standardized variables to eliminate the possibility that mere scale effects might have an effect on the relationships between xylem traits and environmental variables. *Z*-score standardization was applied as follows: for each tree chronology, standardized values were obtained by subtracting the population mean from the original raw data and dividing the difference by the population standard deviation.

We first applied generalized additive models (GAM) to explore the relationships between xylem traits and environmental variables. Generalized additive models are non-linear and non-parametric regression techniques, which do not require a priori functional relationship specifications between dependent and independent variables. In addition, GAM produce link functions to define a relationship between the response variable mean and a smoothed function of each explanatory variable, allowing the effect of one variable to be explored, with all other variables kept equal (Hastie and Tibshirani 1990, Wood 2006). In this case, we used a mixed version of GAM, where a random effect for the intercept value was included in the model to account for repeated measures within the same chronology. Generalized additive model analysis was performed using the 'mgcv' library from the R statistical suite (R Development Core Team 2011). Model script is reported in Table S2 available as Supplementary Data at *Tree Physiology* Online.

The following two data sets were analyzed: *data set 1*, which was the entire data set; and *data set 2*, obtained by selecting observations corresponding to a cambial age (*Age*) >50 years, which served to reduce the effects on conduit size due to the juvenile stem elongation phase (the so-called ontogenetic effect) and to better separate environmental effects on xylem trait variation; see Carrer et al. (2015) for a methodological review.

In GAM analysis, we used mean annual temperature (T_m), total annual precipitation (P), mean temperature (T_g) and total precipitation (P_g) for the period estimated more crucial for tree-ring formation, mean annual nitrogen deposition rate (N_{dep}) and cambial age (*Age*) as covariates in the models. In the random part of the model, a variable was introduced to account for different wood types, i.e. conifer, diffuse-porous and ring-porous species (see Table S2 available as Supplementary Data at *Tree Physiology* Online).

A Bayesian analysis was also performed on *data set 2*. The analysis was conducted as an independent method to evaluate the

relationships between environmental variables and xylem traits. The statistical model used in this analysis was analogous to a multiple regression with standardized by species (centered and scaled) variables. We used a hierarchical approach with a normally distributed random intercept for each tree-ring series, assuming weak prior distributions (mean = 0 and standard deviation = 10^6) and a normal residual error distribution. Markov Chain Monte Carlo simulations were performed with the JAGS program (version 4.0.0; Plummer 2003) running four different chains, each with 40×10^3 'burn-in' cycles and 20×10^3 subsequent cycles with a thinning rate of 1 out of 20 steps. The median, 2.5% and 97.5% quantiles were computed on the posterior distribution of regression coefficients, and a significant relationship was inferred if the inter-quantile interval did not overlap the zero value.

Results

Large xylem trait variation (ring width W_r, conduit area A_c and conduit density D_c) was evident across species (Figure 1 and Figure S4 available as Supplementary Data at *Tree Physiology* Online). Results were as follows: (i) W_r showed similar values in diffuse- and ring-porous species, whereas slightly lower values were found in conifer species; (ii) A_c values were markedly higher in ring-porous species, with mid-range values in diffuse-porous species and lower values detected in conifer species; and (iii) D_c data were only available for hardwood species, which indicated considerably lower values for ring-porous species compared with diffuse-porous species (Figure 1).

Xylem traits did not display evident temporal fluctuations; however, certain trends were evident (Figure 2). A slight tendency in W_r to decline during the second half of last century and increased D_c and A_c was noted over the 20th century; A_c displayed a sharp decline in the last few years of the chronology, in these years most of data were from tree-ring chronologies of diffuse-porous species at a Mediterranean site (Table 1).

We evaluated the relationships between conduit area A_c and density D_c, and additional relationships between A_c, vessel composition ($S = A_c/D_c$), lumen frequency ($F = A_c \times D_c$) and tree-ring width W_r, considering only studies where A_c and D_c measurements were certainly independent of ring width. Pooling all species data, very weak (negative) but significant relationships emerged between A_c and D_c and between A_c and W_r (Figure 3a and b) and the absence of any pattern appeared in the relationships among S, F and W_r (Figure 3c and d).

Generalized additive mixed models were applied to evaluate the relationships between xylem anatomical traits and environmental variables (Figures 4–6). In each figure, GAM plots are reported for *data set 1* (whole data set) and *data set 2* (a data set where observations corresponding to a cambial age >50 years were selected).

A cambial age (*Age*) effect was apparent, particularly due to W_r and A_c in GAM analyses performed on *data set 1* (Table 2). However, after the juvenile phase, no clear variation in xylem traits associated with age was evident and no significant smoothed terms for *Age* were shown by GAM analyses in *data*
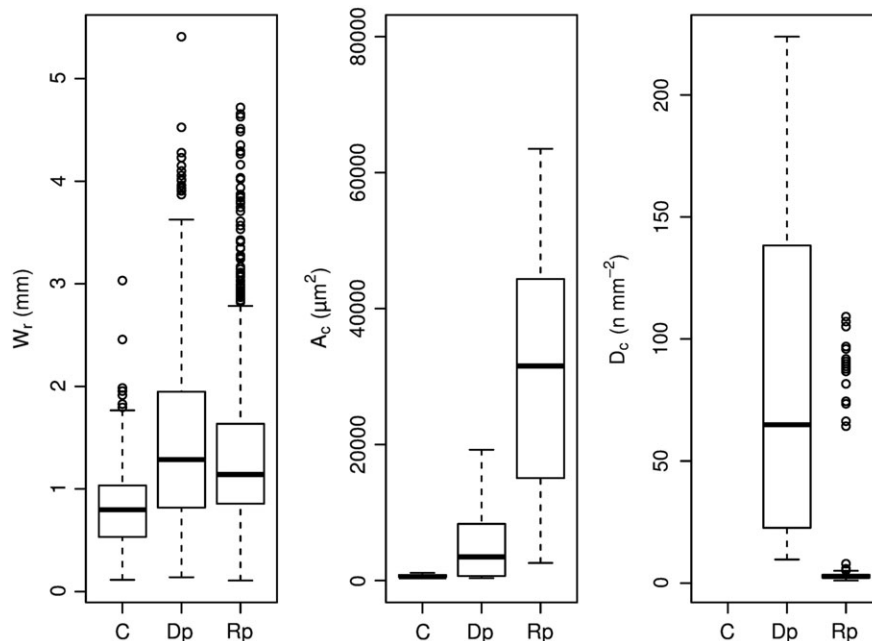
Figure 1. Ring width (W_r) boxplot, conduit area (A_c) and vessel density (D_c) representing xylem traits variability in conifer (C), diffuse-porous (Dp) and ring-porous (Rp) species; each box represents the 75th to 25th percentiles, and the line inside the median; upper and lower marks are the largest to smallest observation values, which are less than or equal to the upper and lower quartile plus 1.5 the length of the interquartile range; circles outside the lower–upper mark range are outliers.
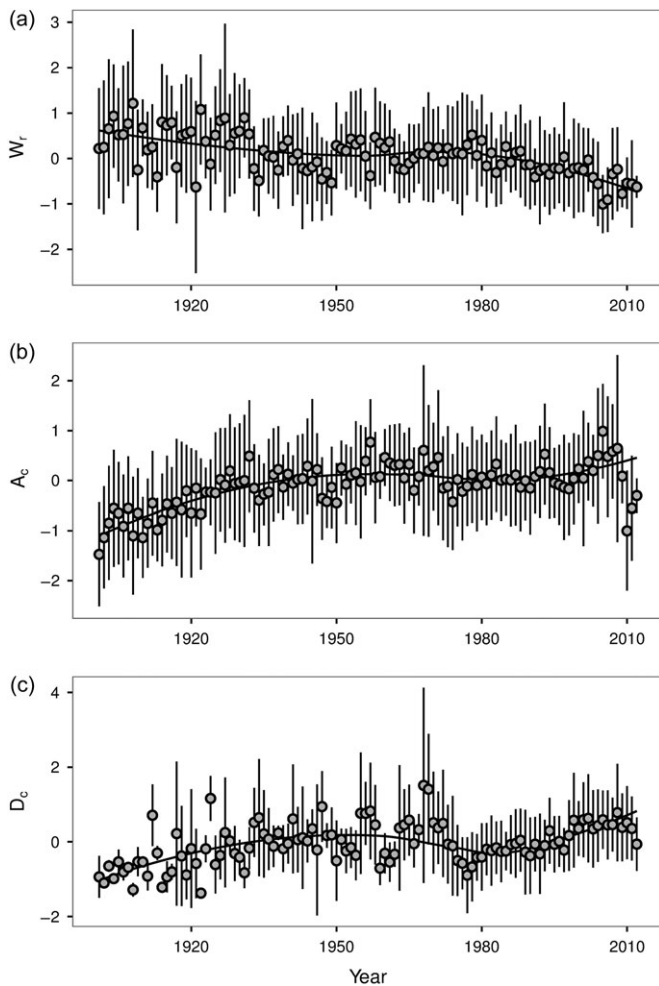
Figure 2. Chronologies of (a) tree-ring width (W_r), (b) conduit size (A_c) and (c) conduit density (D_c), data of all species pooled and averaged for each year. Standardized data are reported on the y-axis, obtained by subtracting the population mean from the original raw data, then dividing the difference by the population standard deviation; vertical bars represent standard deviation. Continuous lines correspond to a locally weighed polynomial regression.

Figure 3. Relationships between: (a) conduit area (A_c) and conduit density (D_c); (b) A_c and ring width (W_r); (c) lumen frequency F (= $A_c \times D_c$) and W_r; and (d) vessel composition S (= A_c/D_c) and W_r. Standardized data are reported, obtained by subtracting the population mean from the original raw data and dividing the difference by the population standard deviation. The linear relationships between A_c and D_c ($y = 0.04-0.26x$, $F_{stat(1,490)} = 35.8$; $P < 0.0001$, $r^2 = 0.06$) and between A_c and W_r ($y = 0.04-0.18x$, $F_{stat(1,552)} = 18.7$; $P < 0.0001$, $r^2 = 0.04$) were statistically significant. The relationships between F and W_r and between S and W_r were not significant. Shaded areas in the upper plots indicate the 95% confidence interval.

set 2 (Table 2 and Figure S5 available as Supplementary Data at *Tree Physiology* Online).

The most appreciable relationships detected by GAM for environmental variables were as follows: (i) W_r exhibited a stable positive relationship with P_g and a negative relationship with N_{dep}, which showed significant smoothed terms in both data sets analyzed (Figure 4c and e, Table 2); (ii) A_c exhibited a positive significant effect for N_{dep} in *data set 2*, and negative P and T_m effects for *data set 2*, in which positive A_c trends were instead observed for T_g and P_g (Figure 5); and (iii) D_c showed a positive effect of N_{dep} and a negative effect of P_g was indicated in both data sets, while positive P and T_g effects were shown only in *data set 1* (Figure 5, Table 2).

A rather different picture emerged when the F (= $A_c \times D_c$) and S (= A_c/D_c) indices were examined. Here, no clear and constant patterns were evident, apart from a negative relationship
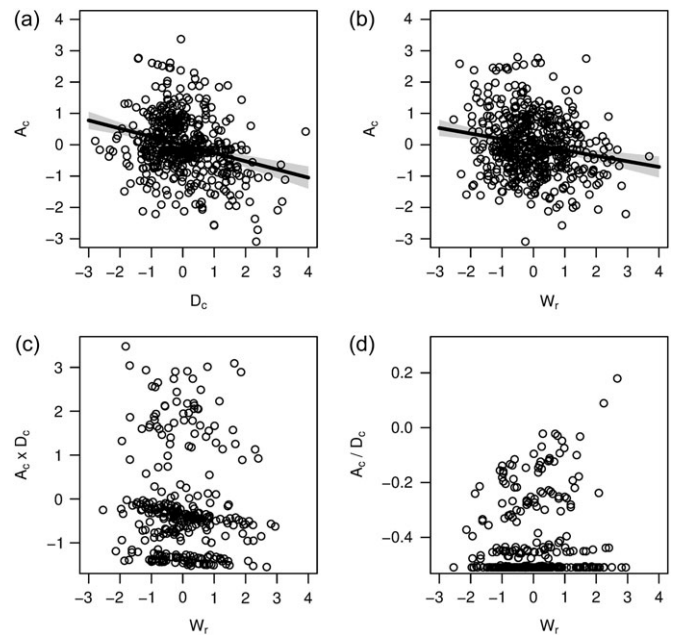
for T_g on F index in *data set 2*, and a negative relationship for N_{dep} on S in both data sets (Figure 6).

The GAM statistics (Table 2) revealed a poor performance of some of the tested models, indeed in 7 out of 10 models the explained variance was lower than 20%. For this reason, we used a Bayesian regression model (on *data set 2*) as an independent statistical approach; this confirmed some of the previous results, as follows: (i) W_r showed a positive effect of P_g and a negative effect of N_{dep}, similar to GAM; (ii) A_c exhibited a positive effect of N_{dep} (comparable to the GAM analysis), whereas no significant effects were detected for other variables; (iii) D_c revealed no significant effects; (iv) F index detected a positive effect of N_{dep} (as in GAM) and T_m; and (v) S index indicated a negative effect of N_{dep} (similar to GAM) (Figure 7).

Discussion

Long-term trends and xylem trait relationships

Long-term radial growth showed an almost flat trend, with a slight tendency to decrease in the second half of the 20th century (Figure 2a). No apparent trace of the fertilization effect was observed in our radial growth data due to the current atmospheric CO_2 rise, consistent with other tree-ring analyses. For
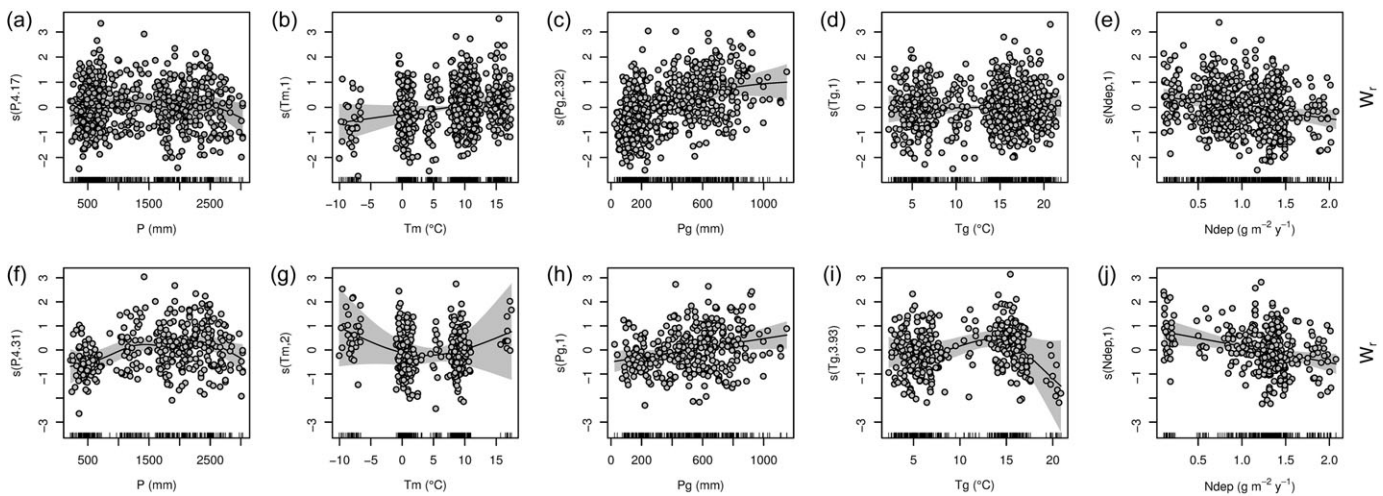
Figure 4. Generalized additive model results of environmental variables for ring width (W_r); cambial age (Age) was also used as covariate in the model. P, total annual precipitation; T_m, mean annual temperature; P_g, total precipitation for the period estimated as more important for ring formation; T_g, mean temperature for the period estimated as more important for ring formation; N_{dep}, annual rate of nitrogen deposition. In the random part of the model, a variable was introduced to account for different wood types, i.e. conifer, diffuse-porous and ring-porous species. Upper row panels: GAM results for *data set 1* (entire data set); lower row panels: GAM results for *data set 2* (observations corresponding to a cambial age >50 years were selected). The y-axis values indicate the x-axis covariate effect on deviation from the mean predicted by the model (continuous line). The shaded areas indicate the 95% confidence interval. The number on each y-axis caption is the effective degrees of freedom for the term plotted. The small lines along the x-axis are the 'rug', showing the observation density. The continuous line is an estimate of the smooth function of the partial residuals (thus the y-axis is centered on zero) and indicates the x-axis covariate effects on the measured trait. In these plots, a positive slope of the continuous line shows a positive effect of the x-variable, and a negative slope of the line indicates a negative effect.

instance, despite the positive CO_2 increase effects on water-use efficiency, enhanced growth patterns were not indicated in worldwide long-term tree-ring analyses (Peñuelas et al. 2011). Comprehensive tree-ring data sets from tropical, temperate and boreal biomes provided supporting evidence that local environmental stress conditions, primarily due to an unfavorable temperature and precipitation balance, can override any potential growth benefits due to increased CO_2 (Battipaglia et al. 2015, Groenendijk et al. 2015, Martin-Benito and Pederson 2015, Zang et al. 2015). In addition, even under suitable environmental conditions, and in dependence on stand structure and concurrence level, height rather than radial growth can represent the preferential carbon destination from enhanced assimilation rates (Domec and Gartner 2003, Rita et al. 2015).

Conduit size (A_c) and density (D_c) displayed a slightly positive increase over most of the time period examined in our study (Figure 2b and c). Conduit size (A_c) and D_c might be considered as functional traits, respectively, related to xylem efficiency and safety; to achieve homeostasis in the conduction area, opposite variation of A_c to D_c could be expected across environmental gradients. However, pooling all species data, A_c and D_c revealed a scattered relationship, with only a modest negative link. Therefore, the hypothesis of an efficient trade-off between these traits was not supported in our data set (Figure 3).

Gleason et al. (2015) recently compiled physiological measurements across a large number of woody plant species with similar conclusions: xylem safety, evaluated as its ability to resist cavitation and embolism; and xylem efficiency, evaluated as its

ability to efficiently conduct water to the leaves were indeed found to be largely independent of one another, suggesting a weak trade-off between these physiological processes (Gleason et al. 2015). At the species level, no clear link was also reported between hydraulic efficiency and xylem mechanical strength throughout a range of angiosperm trees (Zhang et al. 2013).

However, an examination of the product between conduit size and conduit density (the so-called lumen frequency, $F = A_c \times D_c$), and their ratio (the so-called vessel composition, $S = A_c/D_c$) through our long-term tree-ring series could suggest a different picture. In fact, in several cases F and S did not exhibit significant relationships, with the exception of nitrogen deposition (further discussion below), on environmental variables (Figures 6 and 7). It is noteworthy that F represents the relative amount of tree ring covered by water-conducting vessels and increased F values could suggest declined wood mechanical strength due to decreased support tissue (Preston et al. 2006). Vessel composition (S) measures the vessel characteristics within the tree rings; higher S values indicate that the conducting area is comprised of few large vessels, efficient at fast water transport, but potentially at increased risk of embolisms (Tyree and Zimmermann 2002). Overall, conservative F and S values across a wide range of environmental conditions (and also over a large spectrum of radial growth rates, Figure 3) might suggest that a plant trade-off strategy between water transport efficiency, water safety and wood mechanical strength is operating, and might have an effect on future trajectories of xylem features under changing environmental conditions.
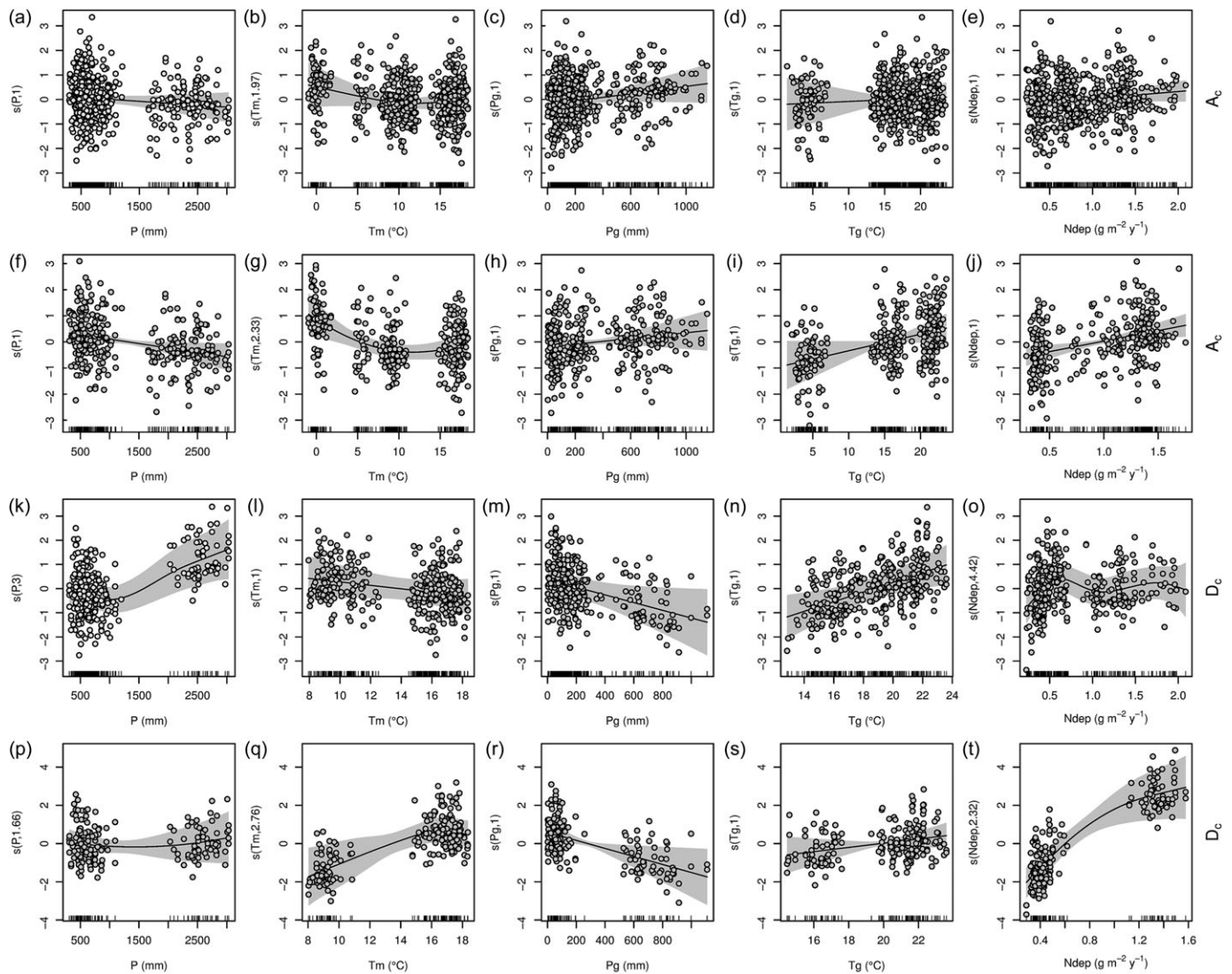
Figure 5. Generalized additive model results of environmental variables for conduit area (A_c, upper two panels) and conduit density (D_c, lower two panels); cambial age (*Age*) was also used as a covariate in the model. *P*, total annual precipitation; T_m, mean annual temperature; P_g, total precipitation for the period estimated as more important for ring formation; T_g, mean temperature for the period estimated as more important for ring formation; N_{dep}, annual nitrogen deposition rate. In the random part of the model, a variable was introduced to account for different wood types, i.e. conifer, diffuse-porous and ring-porous species. For each trait (A_c and D_c), two panels are shown: upper panel, GAM results for data set 1 (entire data set) are shown; lower panel, GAM results for data set 2 (observations corresponding to a cambial age >50 years were selected) are shown. The *y*-axis values indicate the *x*-axis covariate effects on deviation from the mean predicted by the model (continuous line). The shaded areas indicate the 95% confidence interval. The number on each *y*-axis caption is the effective degrees of freedom for the term plotted. The small lines along the *x*-axis are the 'rug', which show the observation density. The continuous line is an estimate of the smooth function of the partial residuals (thus, the *y*-axis is centered on zero) and indicates the *x*-axis covariate effects on the measured trait. In these plots, a positive slope of the continuous line shows a positive effect of the *x*-variable, and a negative slope of the line indicates a negative effect.

The link between the environment and xylem traits

Olson et al. (2014) conducted a recent large-scale investigation across angiosperm species and identified plant size as the main driver of general variability in xylem conduit size, suggesting only an indirect (or residual, after plant size effect was factored out) role of environmental gradients. At a given cambial age, conduit size is dependent on stem elongation rate (the so-called ontogenetic effect), according to well-described patterns of

conduit widening with axial elongation (Anfodillo et al. 2006). Our analysis did not estimate the relationship between cambial age and plant height, which was required to provide a straight-forward separation of environmental from the ontogenetic effect (Carrer et al. 2015). As described in the Materials and methods section, we addressed this point by selecting a data subset (*data set 2*), where juvenile data were excluded. Indeed, GAM analysis did not detect significant smoothed terms for cambial age in this subset (Figure S5 available as Supplementary Data at
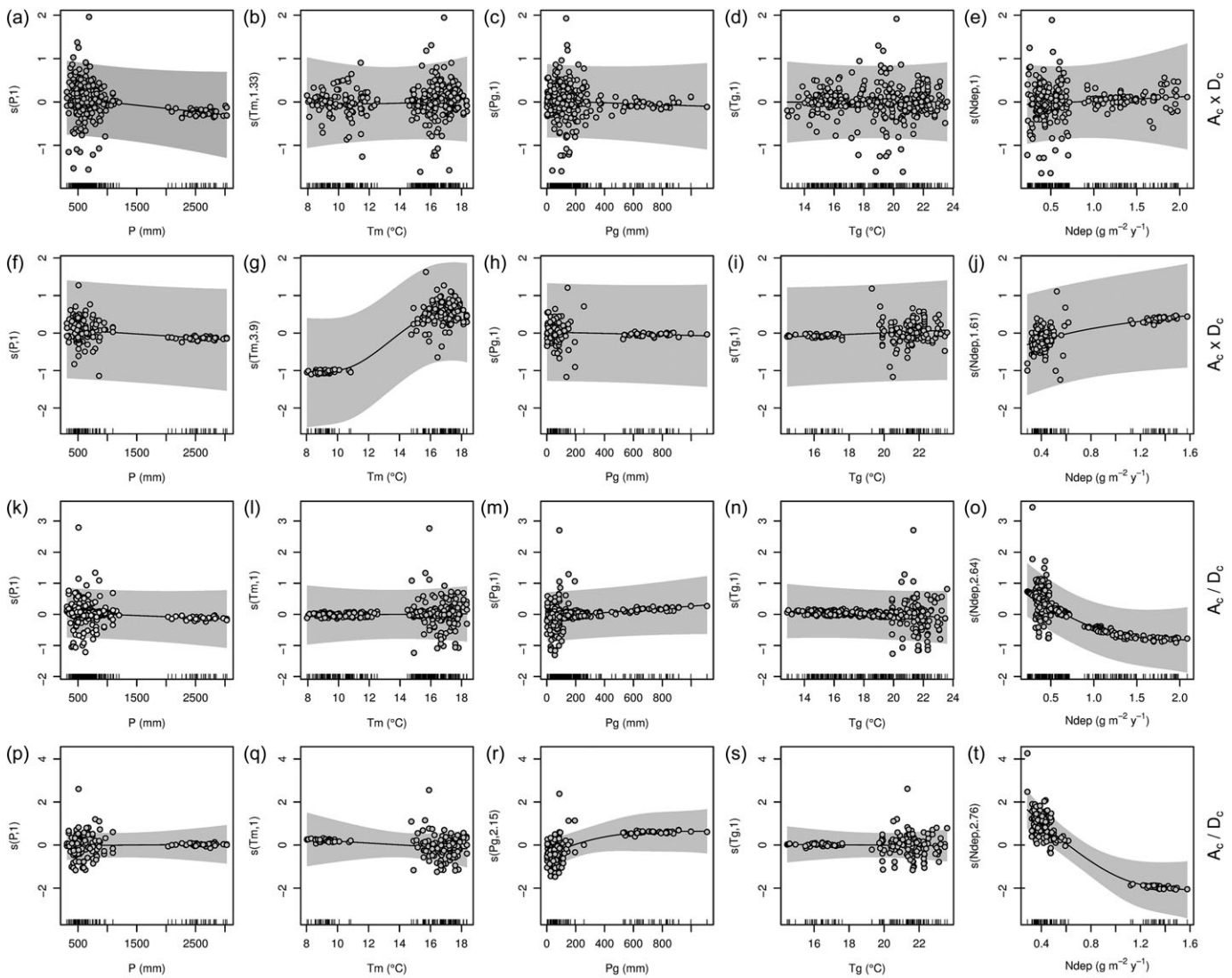
Figure 6. Generalized additive model results of environmental variables for lumen frequency ($F = A_c \times D_c$, upper two panels) and vessel composition ($S = A_c/D_c$, lower two panels), cambial age (*Age*) was also used as a covariate in the model. *P*, total annual precipitation; T_m, mean annual temperature; P_g, total precipitation for the period estimated as more important for ring formation; T_g, mean temperature for the period estimated as more important for ring formation; N_{dep}, annual nitrogen deposition rate. In the random part of the model a variable was introduced to account for different wood types, i.e., conifer, diffuse-porous and ring-porous species. For each trait (*F* and *S*), two panels are shown. The upper two panels depict GAM results for *data set 1* (the entire data set); the lower two panels depict GAM results for *data set 2* (observations corresponding to a cambial age >50 years were selected). The *y*-axis values indicate *x*-axis covariate effects on deviation from the mean predicted by the model (continuous line). The shaded areas indicate the 95% confidence interval. The number on each *y*-axis caption is the effective degrees of freedom for the term plotted. The small lines along the *x*-axis are the 'rug', which show the observation density. The continuous line is an estimate of the smooth function of the partial residuals (thus, the *y*-axis is centered on zero) and indicates the *x*-axis covariate effects on the measured trait. In these plots, a positive slope of the continuous line shows a positive effect of the *x*-variable, and a negative slope of the line indicates a negative effect.

Tree Physiology Online), so we can be rather confident the ontogenetic effect was unlikely to overshadow the environmental signal.

We acknowledge an additional uncertainty potentially affecting the link between environmental gradients and xylem traits in our results; climatic variables and nitrogen deposition rate in our data set were derived from interpolated values on a 0.5° grid square resolution; therefore, the values could diverge from actual climatic conditions where trees were growing. These divergence effects (which would be larger for highly heterogeneous environments) for the observed relationships can hardly be estimated; however,

we might expect some level of additional introduced variability, with the possibility of weakened relationships between environmental variables and xylem functional traits.

Overall, our independent GAM and Bayesian analyses emphasized some significant relationships between xylem functional traits and environmental variables (Figures 6 and 7, Table 2), suggesting some potential for xylem structure to respond to environmental conditions. Here we pay particular attention to cases where significant trends were shown in GAM and Bayesian analyses.

Table 2. Generalized additive models statistics for analyses performed on *data set 1* (entire data set) and *data set 2* (observations corresponding to a cambial age >50 years).

Trait	Variable	Data set 1		Data set 2	
		d.f.	F_{stat}	d.f.	F_{stat}
W_r	*Age*	4.55	4.73***	1.00	0.26
	P	4.17	2.69	4.30	4.03*
	T_m	1.00	2.72	1.99	1.20
	N_{dep}	1.00	6.91**	1.00	8.43**
	P_g	2.31	10.21***	1.00	4.90*
	T_g	1.00	0.06	3.92	6.05***
			$R^2 = 0.14$		$R^2 = 0.15$
A_c	*Age*	6.99	11.46***	2.14	2.48
	P	1.00	1.2	0.99	5.15*
	T_m	1.97	2.46	2.32	3.79*
	N_{dep}	1.00	2.95	1.00	9.14**
	P_g	1.00	2.91	1.00	1.31
	T_g	1.00	0.12	1.00	3.72
			$R^2 = 0.13$		$R^2 = 0.07$
D_c	*Age*	5.70	7.44***	1.00	0.08
	P	3.00	4.88**	1.65	0.89
	T_m	1.00	1.18	2.75	1.92
	N_{dep}	4.42	4.61***	2.31	13.72***
	P_g	1.00	4.60*	1.00	5.74*
	T_g	1.00	8.47**	1.00	1.59
			$R^2 = 0.09$		$R^2 = 0.17$
F	*Age*	1.00	5.50*	1.45	0.40
	P	1.00	1.24	1.00	0.91
	T_m	1.33	0.11	3.89	4.90***
	N_{dep}	1.00	0.08	1.61	1.64
	P_g	1.00	0.13	1.00	0.12
	T_g	1.00	0.00	1.00	0.39
			$R^2 = 0.14$		$R^2 = 0.69$
S	*Age*	4.40	3.32***	1.00	0.80
	P	1.00	0.40	1.00	0.01
	T_m	1.00	0.00	1.00	0.25
	N_{dep}	2.63	8.64***	2.75	9.92***
	P_g	1.00	1.50	2.15	2.74
	T_g	1.00	0.32	1.00	0.01
			$R^2 = 0.29$		$R^2 = 0.66$

The approximate significance of smoothed term *s* is reported, with degree of freedom (d.f.) and F_{stat} statistics. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively. Symbols are as follow: W_r, ring width; A_c, conduit area; D_c, conduit density; *Age*, cambial age; *P*, total annual precipitation; T_m, mean annual temperature; P_g, total precipitation for the period estimated as more important for ring formation; T_g, mean temperature for the period estimated as more important for ring formation and N_{dep}, annual nitrogen deposition rate.

Indeed, rather well-known patterns (Fonti et al. 2010) were reasonably reproduced in our results; for instance the decrease of conduit density with precipitation and its increase with temperature, as well as increased conduit size with precipitation and temperature. Narrow and embolism-resistant conduits have often been reported in cold or drought-prone areas, whereas wide and efficiently conducting conduits prevail in warm and moist areas (Wheeler et al. 2007 and De Micco et al. 2008 among several others).

Conduit size and conduit density relationships with climatic variables do not rule out the hypothesis of coordinated xylem traits along an embolism resistant-conductive efficiency trajectory. In particular, the flat trends in *F* and *S* indices associated with climatic variation supported the hypothesis of an efficient homeostasis in xylem function across a wide range of climatic conditions.

The role of nitrogen deposition

This is the first reported study on the relationships between nitrogen deposition (N_{dep}) and functional xylem traits across a range of tree species and environmental gradients. Generalized additive models and Bayesian analyses results performed on the data sets (where unbalanced distribution of tree functional groups across N_{dep} gradients was not observed, particularly for diffuse-porous and ring-porous species, Figure S3 available as Supplementary Data at *Tree Physiology* Online) was found N_{dep} significantly associated with xylem trait variation: in particular, an effect was evident for conduit density (D_c), which increased with N_{dep}, and consequently, the *S* index exhibited a decrease. It is noteworthy that no evidence has been reported so far that conduit density could depend on stem elongation rate (the ontogenetic effect). In addition, it was notable that N_{dep} was the only variable in both data sets to affect *S*, which otherwise remained unaffected across a wide range of climatic conditions. Bayesian analysis also showed a significant positive effect of N_{dep} on the *F* index; however, no significant pattern emerged from GAM analysis (Figures 4–6).

This result suggested that enhanced *N* availability might favor xylem structure with more vessels per unit surface rather than increased conduit size. This trait can be beneficial for speed water transport, but even more to increase xylem capacity to withstand the risk of runaway cavitation and drought-induced embolism, especially if D_c associated with vessel clustering enables water to more easily bypass air-filled conduits through alternative pathways (Tyree and Zimmermann 2002).

To date, limited results on the effects of increased *N* availability on functional xylem traits are available. The information that does exist comes from manipulative studies on young deciduous trees and depicts a picture rather different than our long-term observational pattern. Indeed, these published studies show increased nitrogen availability results in wider vessels and thinner vessel walls (Watanabe et al. 2008, Plavcová and Hacke 2012, Plavcová et al. 2013), suggesting a xylem more efficient in water conduction, but more vulnerable to drought-induced cavitation.

In our data set, we have to acknowledge that N_{dep} generally increased with time and in most sites was correlated with increasing atmospheric CO_2 concentrations (see Figure S6 available as Supplementary Data at *Tree Physiology* Online); this makes it difficult to identify causal effects and prove that one or the other variable is the real driver of change in functional traits. On the other hand, according to available review, rising CO_2 has a less drastic
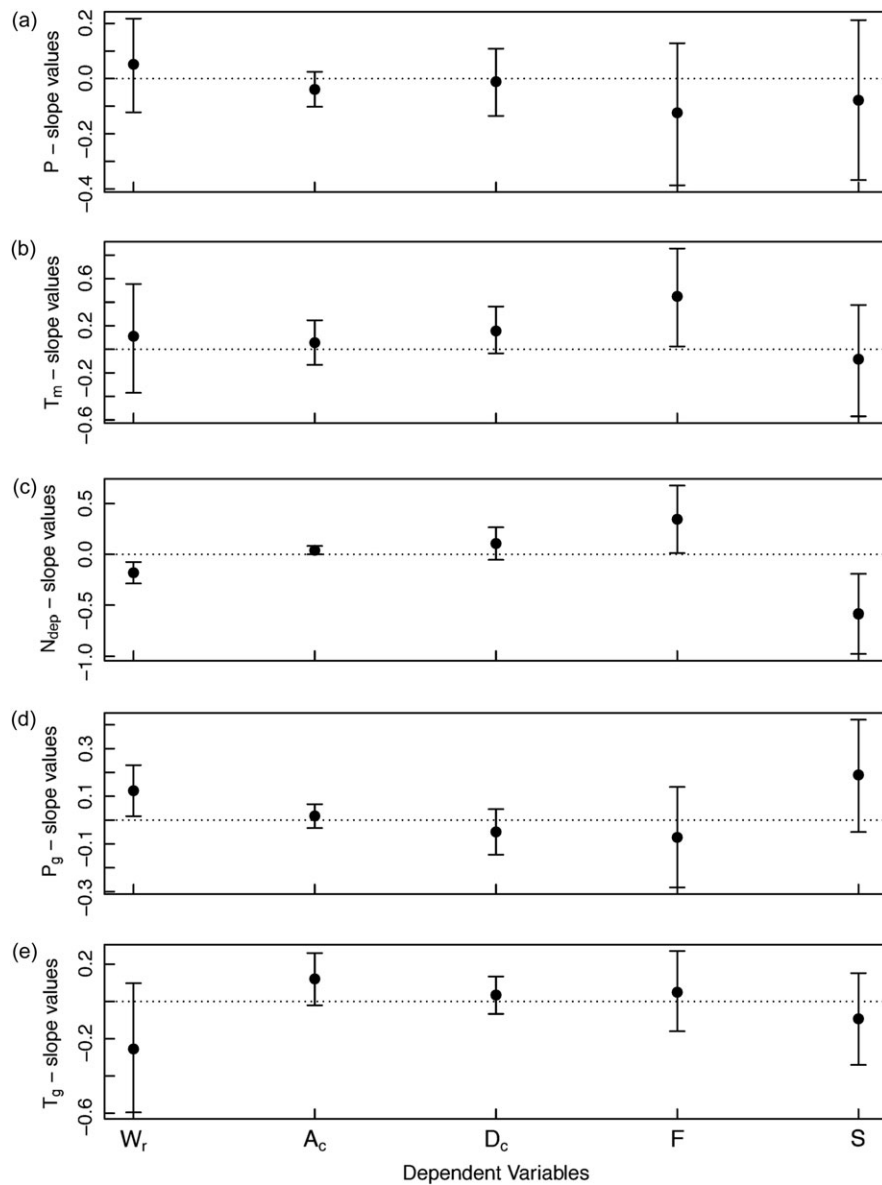
Figure 7. Bayesian analysis results performed on *data set 2*, in which observations corresponding to a cambial age >50 years were selected. Circles are median values for slopes of relationships between environmental variables on the *y*-axis (P, total annual precipitation; T_m, mean annual temperature; P_g, total precipitation for the period estimated as more important for ring formation; T_g, mean temperature for the period estimated as more important for ring formation; N_{dep}, annual rate of nitrogen deposition) and xylem traits on the *x*-axis (W_r, ring width; A_c, conduit area; D_c, conduit density; F, lumen area = $A_c \times D_c$; S, vessel composition = A_c/D_c). Positive and negative values indicate positive and negative effects, respectively. Bars represent the 95% confidence interval, if overlapping the zero line slopes were not significant.

effect on wood anatomical features than on the physiological properties of trees (Yazaki et al. 2005).

Overall, the different scenario emerging from our analysis could be interpreted as the result of a more long-term plant acclimation process. As we previously described, increased N_{dep} did not translate into enhanced radial growth, but rather a negative relationship between radial growth and N_{dep} was observed (Figure 4); however, N_{dep} might affect other carbon-demanding processes, for example height growth or xylem structural adjustment. The latter might be associated with N_{dep} through increased nitrogen availability; if we assume xylem to be cylinder conduits with a fixed length and a constant xylem conduction area, we readily see that the wall surface area of all conduits collectively is proportional to $n^{0.5}$ where n represents the number of xylem conduits. The subsequent hypothesis can be generated: N_{dep} and the associated increase in N availability can elicit plant assimilation, possibly interacting positively with increased atmospheric CO_2 concentrations; in turn, this might provide the carbon investment necessary for additional wall area required to construct xylem with higher conduit numbers per unit surface with an unmodified (or even increased) water conduction area.

The following conclusions might be drawn from our study: first, in some cases our results showed rather clear associations between xylem functional traits and environmental variables, suggesting a potential for xylem to acclimate to environmental conditions. A means to enhance the separation of environmental effects should be derived from future studies. This can be achieved in dedicated experimental research, where long-term variation in xylem anatomical traits is associated with locally measured environmental variables, and tree height-cambial age is properly reconstructed. Second, xylem trait (conduit size and conduit density) response to climatic variables, and particularly the rather homogeneous F and S values, suggested homeostasis in xylem structure across a wide range of climatic conditions, balancing water transport efficiency, water safety and wood mechanical strength. Finally, for the first time, a significant link between nitrogen deposition and xylem functional traits was described from the long-term evidence; N_{dep} was associated with increased conduit numbers per surface unit, a trait in addition to increased conduit size. This depicts conditions where increased nitrogen availability might, at least within the observed N_{dep} range ($<2.5\,\mathrm{g\,m^{-2}\,year^{-1}}$), facilitate construction of a xylem structure efficient for water transport, and concurrently provide enhanced resistance to embolization hazards.

Supplementary Data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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