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Understanding trait interactions and their impacts on growth in Scots pine branches across Europe

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Summary

1. Plants exhibit a wide variety in traits at different organizational levels. Intraspecific and interspecific studies have potential to demonstrate functional relationships and trade-offs amongst traits, with potential consequences for growth. However, the distinction between the correlative and functional nature of trait covariation presents a challenge because traits interact in complex ways.

2. We present an intraspecific study on Scots pine branches and use functional multi-trait concepts to organize and understand trait interactions and their impacts on growth. Branch-level traits were assessed for 97 branches from 12 Scots pine sites across Europe.

3. To test alternative hypotheses on cause–effect relationships between anatomical traits, hydraulic traits and branch growth, we measured for each branch: the tracheid hydraulic diameter, double cell wall thickness, cell lumen span area, wood density, cavitation vulnerability, wood-specific hydraulic conductivity, the leaf area to sapwood area ratio and branch growth. We used mixed linear effect models and path models to show how anatomical traits determine hydraulic traits and, in turn, how those traits influence growth.

4. Tracheid hydraulic diameter was the best predictor of cavitation vulnerability ($R^2 = 0{\cdot}09$ explained by path model) and specific conductivity ($R^2 = 0{\cdot}19$) amongst anatomical traits. Leaf area to sapwood area ratio had the strongest direct effect on branch growth ($R^2 = 0{\cdot}19$) and was positively associated with the tracheid hydraulic diameter ($R^2 = 0{\cdot}22$). A number of bivariate correlations between traits could be explained by these functional relationships amongst traits.

5. The plasticity in tracheid hydraulic diameter (10.0–15.1 μm) and leaf area to sapwood area ratio (600–6051 $\text{cm}^2\ \text{cm}^{-2}$) and the maintenance of a minimum leaf water potential (between −2 and −2·5 MPa) appear to drive the anatomical and hydraulic traits of Scots pine across Europe. These properties are major drivers of the functional trait network underlying the growth variation amongst pine branches and thus possibly contribute to the ecological success of pines at a local and continental scale.

Key-words: cavitation resistance, cavitation vulnerability, functional trait, growth, P_{50}, implosion, specific conductivity, wood anatomy, wood density, Ψ_{50}

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Introduction

Plants exhibit a wide variety in traits at different organizational levels. Plant trait covariation across species is often interpreted in terms of trade-offs and functional relationships with potentially large consequences for growth, survival or reproduction (Westoby *et al.* 2002; McGill *et al.* 2006; Sterck *et al.* 2011). It has also been suggested that the use of intraspecific variation to demonstrate functional trade-offs is superior to, or at least as valuable as, the use of interspecific variation (cf., Futuyma & Moreno 1988). However, the distinction between the correlative or functional nature of intraspecific trait covariation presents another challenge because traits may interact in complex ways (Martínez-Vilalta *et al.* 2009; Fichot *et al.* 2010). Moreover, intraspecific studies are still scarce (but see, Alder, Sperry & Pockman 1996; Cornwell *et al.* 2007; Choat, Sack & Holbrook 2007) and we cannot yet generalize on intraspecific functional trends, except maybe for some trends associated with tree height (Ryan, Phillips & Bond 2006; Sterck & Schieving 2011).

We have previously suggested that Scots pines (Fig. 1) adjust their branch hydraulic system to climate dryness across Europe by modifications in the leaf area to sapwood area ratio and stomatal control, but without clear acclimation in anatomical (tracheid diameter, cell wall thickness, wood density) or hydraulic traits (cavitation vulnerability, specific hydraulic conductivity) (Martínez-Vilalta *et al.* 2009). Moreover, we showed that pines on drought exposed sites in Spain and Switzerland maintained broadly similar minimum leaf water potentials (−2 to −2·5 MPa, Martínez-Vilalta *et al.* 2009). Such minimum potentials were not found for pines in Scotland (−1·3 MPa) and Finland (−1·9 MPa), probably owing to the much wetter site conditions there (Martínez-Vilalta *et al.* 2009). The maintenance of minimum water potentials is consistent with the strong stomatal control of the hydraulic status under water-stressed conditions (Zweifel, Steppe & Sterck 2007) and the homoeostasis of water transport (Magnani, Grace & Borghetti 2002; Duursma *et al.* 2008). Despite the lack of anatomical acclimation to climate dryness, anatomical as well as hydraulic traits showed substantial variation within and across the same pine populations across Europe (Martínez-Vilalta *et al.* 2009). In this study, we analyse the same data at the individual branch level, instead of site averages, while controlling for site effects. This approach allows us to test for possible cause/effects networks amongst anatomical, hydraulic, structural and growth traits within branches.

Fig. 1. A 70-year-old Scots pine on a drift sand area in the Veluwe forest, central Netherlands (photograph by Leo Goudzwaard).

We start from a conceptual model that explains cavitation vulnerability and specific hydraulic conductivity based on anatomical traits, and distinguish between correlative and directional relationships (double- and single-headed arrows, respectively, Fig. 2a).This model is based on several assumptions. We assumed that tracheid hydraulic diameter and cell wall thickness are correlated, because both traits result from cell expansion. We also expected that tracheid hydraulic diameter and cell wall thickness determine wood density and the ratio of double cell wall thickness to lumen span diameter,

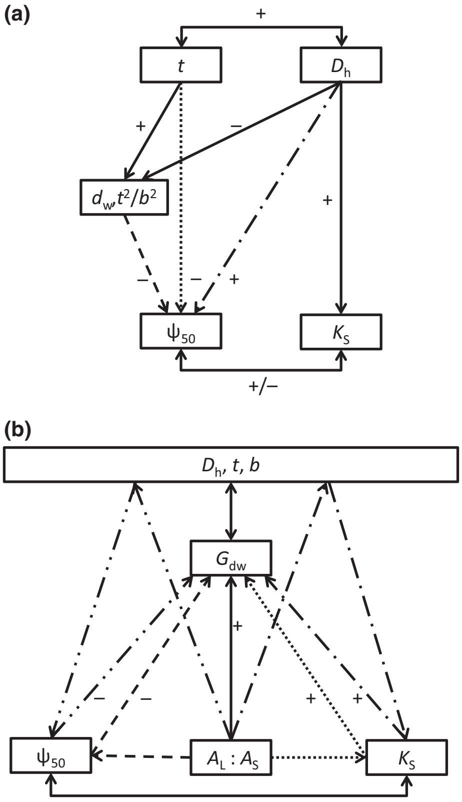

Fig. 2. The hypothesized networks for correlative and functional responses amongst functional traits (for trait symbol explanations, see Table 1). Correlative relationships are indicated by double-headed arrows, and functional relationships are indicated by single-headed arrows. The relationships assumed in any model are indicated by solid lines, while alternative functional relationships are indicated by dashed, dot-dashed, dotted or double-dot-dashed lines. (a) the hypothesized effects of anatomical variables on cavitation vulnerability and specific conductivity; (b) the hypothesized effects of anatomical and hydraulic traits on growth. The relationships with significant anatomical traits (see a) are for simplicity grouped in one box. Signs refer to an expected positive (+) or negative (−) effect. For explanation see text.

hence referred to as thickness-span ratio. Moreover, it is well established that tracheid hydraulic diameter influences the specific conductivity directly (Zimmermann 1983), and indirectly because wide tracheids are longer (Mencuccini, Grace & Fioravanti 1997; Sperry, Hacke & Pittermann 2006) such that less pit membranes (resistances) are encountered per unit water transport length (not shown in Fig. 2). These relationships were assumed in our functional trait network (represented by continuous lines), when explaining specific conductivity from anatomical traits (Fig. 2a).

In addition to these assumptions, we tested alternative, more controversial, cause/effect relations between anatomical traits and cavitation vulnerability (interrupted lines, Fig. 2a). For conifers, there is increasing evidence that cavitation occurs when a pit membrane torus fails to seal the pit aperture and, in turn, air bubbles are seeded into tracheids through pores in the margo (Cochard 2006; Delzon *et al.* 2010). Because greater margo flexibility and greater torus overlap with the pit aperture allow better sealing of the pit aperture, they are considered key traits in controlling cavitation vulnerability. In our study, we only tested for possible effects of tracheid diameter (dot-dashed line Fig. 2a), cell wall thickness (dotted line, Fig. 2a) and thickness-span ratio or wood density (dashed line, Fig. 2a). It was hypothesized that larger tracheids are more vulnerable to cavitation because cavitation occurs because of air seedling via pit membrane pores when pit aperture sealing fails (Cochard 2006; Delzon *et al.* 2010), and either pit pore size increases with cell expansion (Martinez-Vilalta & Pinol 2002) or because the probability of encountering larger pores increases with tracheid diameter (Wheeler *et al.* 2005; Christman, Sperry & Adler 2009; but see Pittermann *et al.* 2006b). Alternatively, it was hypothesized that branch tracheid cells have a higher thickness-span ratio (or higher wood density) when they are less vulnerable to cavitation. The underlying idea is that branches with lower cavitation vulnerability are adapted to, and experience, lower leaf water potentials, but require a larger thickness-span ratio to maintain safety margins against tracheid implosion (Hacke *et al.* 2001). We also tested for direct effects of cell wall thickness on cavitation vulnerability (dotted arrows in Fig. 2a), as shown for different angiosperm genotypes (Fichot *et al.* 2010) and species (Cochard *et al.* 2008). It has been argued that greater cell wall thickness relates to pit membrane thickness and reduced cell porosity (Jansen, Choat & Pletsers 2009), but it is unclear whether such mechanisms also act in conifers such as pine. We thus disentangled alternative hypotheses for the effects of anatomical traits on cavitation vulnerability across pine branches.

In the next step, we analyse how the anatomical and hydraulic trait network affected branch growth (Fig. 2b). As determinants of growth, we first considered the leaf area to sapwood area ratio, because it is a major driver of functional trait variation, being highly plastic in pines (Martínez-Vilalta *et al.* 2009) and because a large ratio implies a larger leaf area driving growth (see also McDowell *et al.* 2008; Sterck *et al.* 2008). We also assumed that growth and tracheid diameter were correlated positively, because rapid growth is usually associated with rapid cell expansion. On top of these assumed relationships (solid lines, Fig. 2a), we explored the role of four alternative pathways for the direct effects of anatomical/hydraulic trait on growth (different interrupted-line networks in Fig. 1b). We tested for direct positive effects of specific conductivity on growth (Fig. 2a), because a higher conductivity allows for higher crown stomatal conductance and photosynthesis. We combined these effects with a possible direct effect of leaf area to sapwood area ratio on specific conductivity (dotted lines, Fig. 2a), or possible indirect effects via anatomical traits (dot-dashed lines, Fig. 2a). We also tested for a direct negative impact of cavitation vulnerability on growth, because a higher cavitation vulnerability may reduce crown stomatal conductance and photosynthesis at low leaf water potentials. We combined this prediction with possible direct effects of leaf area to sapwood area ratio on cavitation vulnerability (dashed lines, Fig. 2a), and indirect effects via anatomical traits (double-dot-dashed lines, Fig. 2a).Using both step 1 (Fig. 2a) and step 2 (Fig. 2b), we thus evaluate the most plausible anatomical/hydraulic functional trait network that helps to explain the observed variation in growth amongst pine branches.

Materials and methods

Branches of pines from tree populations of 12 sites across Europe were studied, ranging from Finland to Spain. The populations occurred along a range of environmental conditions across the distribution of Scots pine (for details: Martínez-Vilalta *et al.* 2009). These sites include the same 12 sites studied by Martínez-Vilalta *et al.* 2009, but include one extra population at Salgesch and one at Pfynwald (both sites in the Swiss Alps).

From each population, a mean number of seven branches (range 5–11) were sampled for this study. The sampled branches were fully exposed, > 40 cm long and 0·5–1 cm in xylem diameter (> 3 year of age). After collection, branches were kept in wet towels to prevent dehydration. In the laboratory, needles were stripped of and total projected leaf area A_l was measured (Martínez-Vilalta *et al.* 2009). The vulnerability to xylem cavitation was measured by the cavitron technique (Cochard *et al.* 2005) within 2 days of sample collection (cf., Martínez-Vilalta *et al.* 2009). Samples were cut in the air to obtain 0·28-m-long segments. The bark was removed from those segments before they were placed in the centrifuge. The xylem pressure was first set at a reference pressure (−1 MPa) and the maximum conductivity (kg m.s^{-1} MPa^{-1}) was measured. Reference pressure was set to a more negative value to determine the new conductivity. This procedure was repeated for more negative pressures, using (−0·5 MPa) step increments, until more than 90% of the maximum conductivity was lost. This technique allows us to estimate the per cent loss of xylem conductivity versus the xylem pressure (for details, see Martínez-Vilalta *et al.* 2009). The negative pressure causing 50% loss of conductivity Ψ_{50} (MPa) was considered a proxy for vulnerability to cavitation. The specific conductivity (K_S in kg m^{-1} s^{-1} MPa^{-1}) was calculated by dividing the maximum hydraulic conductivity by the xylem cross-sectional area of the stem segment. All measurements were analysed in the same laboratory by a single person, to reduce noise in the data.

The same samples were used for the anatomical measurements. Micro thin sections (*c.* 25 μm thick) were taken, following the procedure described in Martínez-Vilalta *et al.* (2009). The sapwood area

A_s was estimated from the xylem cross-section area, in which we corrected for the area occupied by the pith. Leaf area to sapwood area ratio was calculated as $A_l : A_s$. Oven-dry weights of segments were determined after drying at 103 °C. For each sample, fresh wood volume was determined by the water displacement method as described by Olesen (1971). Wood density d_w (kg m^3) was obtained by dividing oven-dry weight by the sample volume. Branch growth rate G_{dw} was calculated as the product of the average annual basal area increment and wood density (kg m^{-1} year^{-1}) and thus represents the estimated annual biomass growth per metre segment length.

The tracheid hydraulic diameter D_h was calculated, assuming elliptical tracheid lumens (Lewis & Boose 1995):

$$D_h = \sqrt[4]{\frac{1}{4}\sum_{i=1}^{n}\frac{2.a_i^3.b_i^3}{a_i^2 + b_i^2}}$$

Where a_i and b_i are the minimum and maximum lumen diameter, respectively, of the *i*th elliptical tracheid, and *n* is the number of tracheids measured.

To estimate the resistance against implosion, we selected 25 pairs of tracheids per sample. Those tracheids were selected such that their diameters were close to the mean hydraulic diameter of the same segment (maximum deviation of 2 μm). For one cell of each pair, we measured the lumen diameter or span area *b* and perpendicular to this span, we measured the double cell wall thickness of the cell pair *t*, according the methods described by Hacke and colleagues (Hacke *et al.* 2001; Hacke, Sperry & Pittermann 2004). The 25 *b* and *t* values were averaged per sample and, together with the corresponding $(t/b)^2$ ratio, used in the statistical analyses.

STATISTICAL ANALYSES

The functional trait variables in our analysis are summarized in Table 1. All variables were checked for normality and transformed by logarithms whenever required, or by a square root in the case of branch growth G_{dw}. Linear mixed-effect models were used to test for the effects of anatomical traits (t, D_h, d_w, $(t/b)^2$) on Ψ_{50} and K_S, as well as for the possible effects of the Ψ_{50}, K_S and $A_l : A_s$ on G_{dw}. In all models, site was included as a random factor affecting intercept and slope of the relationships. Non-significant explanatory variables were removed from the model, if this improved the fit evaluated by the AIC. Linear mixed-effect models were analysed using lme-routines in *R*.

Table 1. Functional plant traits measured or calculated for Scots pine branches ($N = 97$) from 12 sites across Europe

Abbreviation	Explanation	units
$A_l : A_s$	Leaf area to sapwood area ratio	(–)
b	Tracheid lumen span area	(μm)
D_h	Tracheid hydraulic diameter	(μm)
d_w	Wood density	(kg m^{-3})
G_{dw}	Branch growth rate	(kg m^{-1} year^{-1})
K_l	Leaf-specific conductivity	(kg m^{-1} s^{-1} MPa^{-1})
K_S	Specific conductivity	(kg m^{-1} s^{-1} MPa^{-1})
t	Double cell wall thickness	(μm)
$(t/b)^2$	Thickness-span-ratio	(–)
Ψ_{50}*	Xylem water potential at 50% conductivity loss	(MPa)

*Negative values are given.

We employed path analysis (structural equation modelling without latent variables), which allows for testing cause/effect models including more than two traits, as presented in Fig. 2. Path analysis provides the strengths of each hypothesized directional relationship (the single-headed arrows in Fig. 2) and of each hypothesized correlative relationship (double-headed arrows) by path coefficients, which give the relative effect sizes (similar to standardized partial regression coefficients).The validity of the whole model is estimated from the collected covariance matrix amongst the variables using a χ^2 test, which assesses the discrepancy between sampled correlations and correlations implied by the model. The degrees of freedom in this test depend on the number of restrictions in the model, that is, the omitted direct effects (e.g. for t on K_S in Fig. 2a). The χ^2 value and degrees of freedom provide the probability (significance) that the sample correlations differ from implied population correlations, where a low, non-significant χ^2 value confirms a good match between sampled data and model (no difference).

We used path analysis to test the alternative correlative/functional trait networks for the effects of anatomical traits on hydraulic traits (Fig. 2a), as well as the effects of anatomical and hydraulic traits on branch growth (Fig. 2b). The model fit to the data was compared amongst alternative models, where a better model fit was indicated by lower χ^2 values and corresponding non-significant P levels, as well as by a lower AIC value. Path analyses did not account for random site effects, which were considered instead in the mixed-effect models. Path analyses were carried out using PASW-Statistics, AMOS 18·0.

Results

Functional traits varied considerably between and within sites (Fig. 3). Most variables showed considerable overlap in the observed trait values across sites. The strongest differences across sites were observed for branch growth (Fig. 3b), whereas the anatomical trait values overlapped amongst most sites (Fig. 3a). Trait correlations are shown in Table 2.

Mixed-effect models were used to show the effects of anatomical traits on cavitation vulnerability and specific conductivity (Table 3). Site was included as a random factor affecting the intercept of the relationships. In most cases, the model fit was worse (in terms of AIC) if random effects on the slope were also included. Only for the relationship between Ψ_{50} and t did the model improve if random effects on the slope were included. Specific conductivity increased with tracheid hydraulic diameter and tended to decrease with wood density but this was only marginally significant (Table 3). Cavitation vulnerability increased with greater tracheid hydraulic diameter and decreased with higher wood density, and these trends were most significant when accounting for random site effects. Cavitation vulnerability increased with double cell wall thickness, but only became significant when interactive random site effects were included. Regarding the effects of hydraulic and structural traits on growth, specific conductivity had no significant effect (Table 4). Both leaf area to sapwood area ratio and cavitation vulnerability had positive effects on branch growth (Table 4).

A comparison between alternative path models suggested that cavitation vulnerability and specific conductivity were mainly driven by tracheid hydraulic diameter, and not by any of the other anatomical traits (Figs 4a and 5), albeit with a

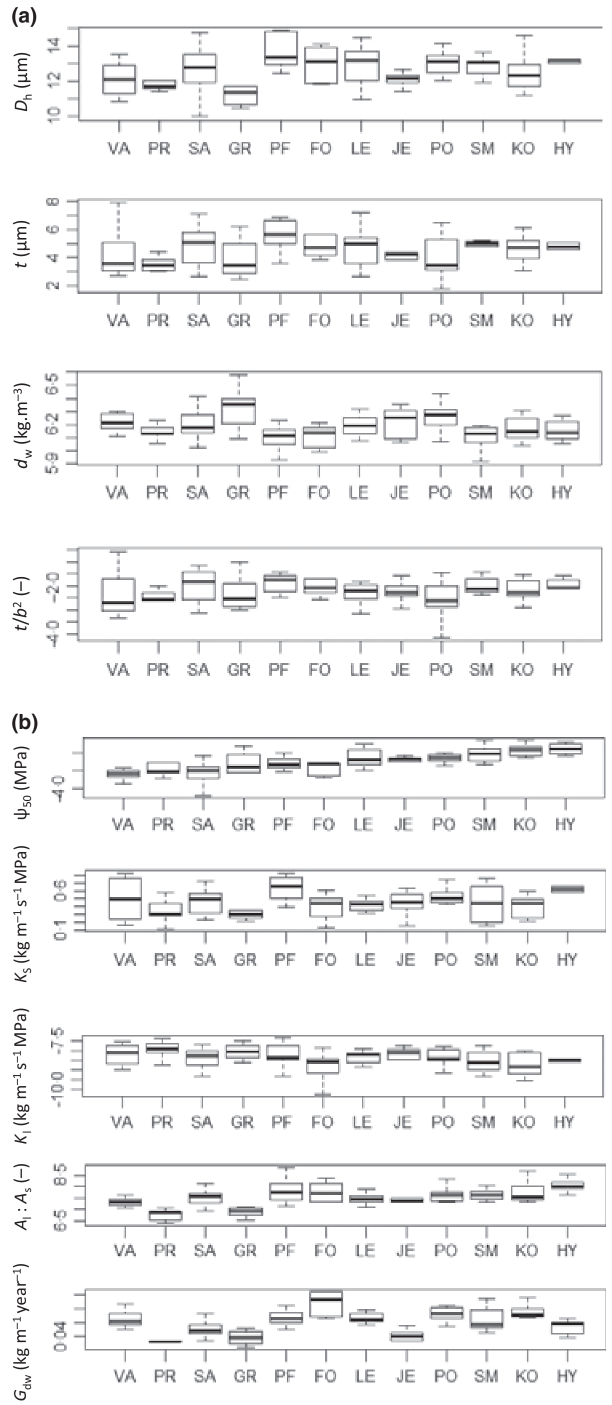

Fig. 3. The variation in functional traits within and across sites. The sites are located in boreal, temperate and Mediterranean areas (see Martínez-Vilalta *et al.* 2009): (a) for anatomical traits, (b) for physiological, structural and growth traits. Boxplots show the 0.1, 0.25, 05, 0.75 and 0.9 quantiles per trait per site. For trait code explanation and units, see Table 1. A_l:A_s, d_w, K_l and $(t/b)^2$: ln-transformed values; G_{dw}: square root transformed, as used in the analysis.

very large scatter in the data. This result implies that the significant effects of wood density on cavitation vulnerability in the mixed-effect models were indirect and caused by its relationship with tracheid hydraulic diameter (Fig. 4a, Table 2). There was a positive effect of cell wall thickness on cavitation vulnerability, but this was marginally significant (Table 3). The second path model suggested that growth was driven directly only by the leaf area to sapwood area ratio (Figs 4b and 6). Moreover, the model confirmed the expected coupling between tracheid hydraulic diameter and growth. The model also implied that the effect of cavitation vulnerability on growth, as suggested by mixed-effect models, was only indirect and resulted from the coupled variation with tracheid hydraulic diameter. The coupled variation between specific conductivity and tracheid hydraulic diameter was, however, not manifested by significant correlations, or effects, between specific conductivity and growth (Tables 2 and 4). Overall, the variation of dependent or exogenous variables explained was lower than in the mixed-effect models (Tables 3 and 4), because random effects were not accounted for in the path analysis.

Discussion

We investigated how branches of Scots pines, collected from 12 sites widely distributed across Europe, coordinated their anatomical, physiological and structural traits, and how these traits in turn affected branch growth. We found a central role of tracheid hydraulic diameter and leaf area to sapwood area ratio in explaining the variation in cavitation vulnerability and specific conductivity across branches. The tracheid hydraulic diameter and leaf area to sapwood area were also directly coupled to branch growth, but cavitation vulnerability or specific conductivity were not. Cavitation vulnerability did not (negatively) impact growth because it was driven by a larger tracheid hydraulic diameter and leaf area sapwood area ratio, which in turn resulted in faster growth. Specific conductivity did not influence growth but contributed to a relatively constant leaf-specific conductivity. Our analysis suggests that tracheid hydraulic diameter plays a central role in the cavitation vulnerability, specific conductivity and growth, rather than the other measured anatomical traits (wood density, cell wall thickness and thickness-to-span ratio). Starting from multi-trait cause–effect models, our analyses thus provided new insights into how apparent trade-offs between anatomical and hydraulic traits with growth may function.

Our results show that tracheid hydraulic diameter indeed drives the variation in specific conductivity across pines (Figs 4a and 5), but the amount of unexplained variation is nevertheless large. The increase in specific conductivity with tracheid hydraulic diameter is in line with the law of Hagen–Poiseuille, which predicts that tracheid conductivity scales with the fourth power of tracheid diameter. Moreover, wider tracheids are longer (Mencuccini, Grace & Fioravanti 1997), and the amount of encountered pits per unit transport length is therefore lower (Sperry, Hacke & Wheeler 2005). Overall, the large variation in the relationship of tracheid hydraulic diameter with specific conductivity relationship may partially be attributed to differences in tracheid density and also in pit structure, which were not explicitly considered but may contribute $>60\%$ to the resistivity ($=1/$conductivity) for water flow in conifer sapwood (Pittermann *et al.* 2006a).

Table 2. Bivariate relationships amongst the functional traits measured and calculated

	D_h	b	t	WD	t^2/b^2	Ψ_{50}	K_S	$A_L : A_S$	K_l	G_{dw}
D_h		***0·87***	ns	***−0·57***	*−0·20*	***0·36***	***0·37***	***0·44***	**ns**	***0·56***
b			ns	***−0·51***	*−0·27*	*0·30*	***0·32***	***0·36***	ns	*0·56*
t				ns	***0·93***	ns	ns	ns	ns	*0·21*
WD					ns	*−0·30*	*−0·23*	*−0·29*	ns	***−0·37***
t^2/b^2						ns	ns	*0·24*	ns	ns
Ψ_{50}							ns	*0·26*	ns	*0·27*
K_S								*0·24*	***0·60***	Ns
$A_L : A_S$									***−0·61***	***0·44***
K_l										*−0·25*
G_{dw}										

Pearson correlation coefficients are presented. For trait codes, see Table 1. $P > 0·05$ is not significant ns, $P < 0·05$ in *italics*, $P < 0·01$ adding *underlined*, $P < 0·001$ adding ***bold***.

Table 3. Linear mixed-effects models linking a given explanatory, anatomical variable with xylem water potential at 50% conductivity loss (ψ_{50}) or specific conductivity (K_S)

Explanatory variable	ψ_{50} effect size	R^2	K_S effect size	R^2
D_H	0·089(0·020)***	0·47	0·057 (0·016)***	0·14
t	0·045 (0·022)*	0·41	–	
$(t/b)^2$	–		–	
d_w	−0·82 (0·21)***	0·45	−0·29 (0·17)$^+$	0·07

The values give the estimated coefficient of the fixed effect and its SD (in brackets). Model fit worsened (in terms of AIC) if random site effects on the slope were also included, except for the relationship between ψ_{50} and t the model improved if random effects on the slope were included. The given coefficients correspond to the best model. Only significant relationships are given, and asterisks indicate the significance level ($^+0·1 > P > 0·05$; $*P < 0·05$; $***P < 0·001$). The explained variation is provided by the R^2 value (Magee 1990) but is in some cases, seriously inflated by the random site effects. In those cases, the R^2 can be much higher than for the bivariate correlations (Table 2) or the variation explained of exogenous (dependent) variables in the path analyses (Fig. 4).

Table 4. Linear mixed-effects models linking the physiological hydraulic traits (ψ_l, K_S) and a structural hydraulic trait ($A_l : A_s$) with a proxy of branch growth (G_{dw}), calculated as the product of radial growth and wood density

Explanatory variable	G_{dw} effect size	R^2
$A_l : A_s$	0·017 (0·0036)***	0·19
K_S	–	
Ψ_{50}	0·017 (0·0053)**	0·41

The values give the estimated coefficient of the fixed effect and its SD (in brackets). Site was included as a random factor affecting the intercept of the relationships. In all cases, the best model fit (in terms of AIC) was obtained if random effects on the slope were not included. Only significant relationships are given, and asterisks indicate the significance level ($**P < 0·01$, $***P < 0·001$). The explained variation is provided by an R^2, but note the remarks on this (Table 3 legend).

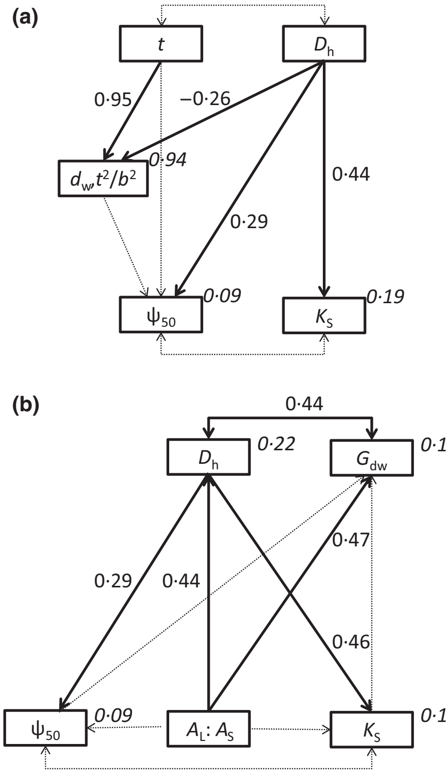

Fig. 4. The results of the path model analysis, showing the most significant of the alternative, hypothesized, models (Fig. 2, and Introduction section) for, (a), effects of anatomical variables on cavitation vulnerability and specific conductivity and, (b), the effects of anatomical and hydraulic traits on growth. For statistics, see Table 5. The values along the arrow indicate standardized coefficients, and the italic values at top right corner of exogenous variables refer to the amount of variation explained by the model for such variables. For the trait symbol explanations, see Table 1. Thick lines represent significant relationships, narrow lines stand for non-significant relationships.

The relationship between anatomical traits and cavitation vulnerability is more controversial. Our results show that tracheid diameter is a better predictor of cavitation vulnerability than wood density, (double) cell wall thickness or thickness-to-span ratio (Fig. 4a). Based on the recent support for the role of pit properties in cavitation vulnerability in conifers, we might argue that the tracheid hydraulic diameter simply acts as a correlate of the pit properties, which is indeed true across conifer species (Delzon *et al.* 2010). Alternatively, it can be speculated that greater cavitation vulnerability in branches with larger tracheid diameters may be caused by wider pits

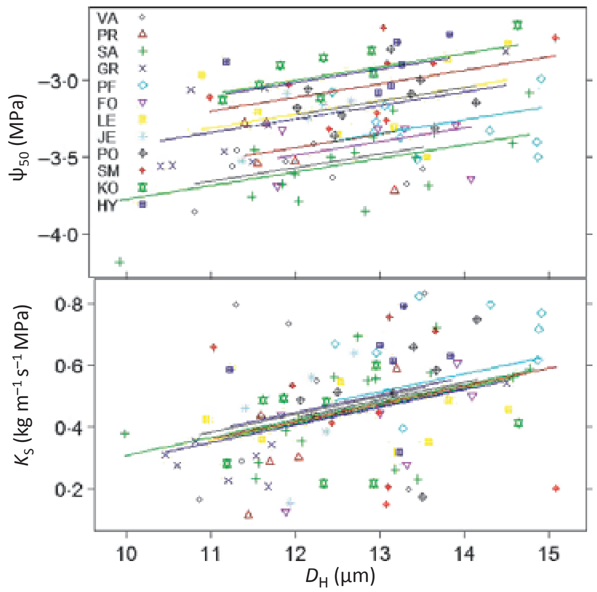

Fig. 5. The significant functional relationships of the path models illustrated with the results from the linear mixed-effect models of Table 3. The effects of tracheid hydraulic diameter on cavitation vulnerability and specific conductivity (see also Fig. 4a). For trait symbol explanation, see Table 1. Sites are indicated by different symbols (see Martinéz-Vilalta *et al.* 2009).

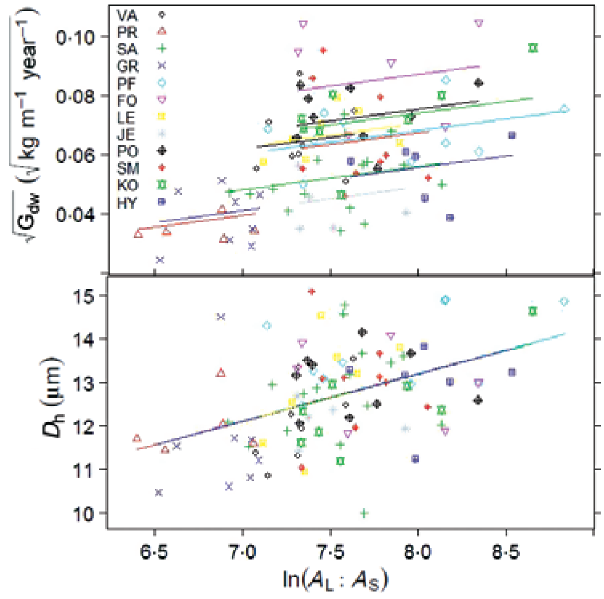

Fig. 6. The significant functional relationships of the path models illustrated with the results from the linear mixed-effect models of Table 4. The effects leaf area to sapwood area ratio on growth and tracheid hydraulic diameter (see also Fig. 3b). For trait symbol explanation, see Table 1. Sites are indicated by different symbols (see Martinéz-Vilalta et al. 2009).

and larger pores in pit membranes resulting from greater cell expansion (Martínez-Vilalta *et al.* 2002), or by the greater probability of encountering wider pits and large pores in larger cells (Wheeler *et al.* 2005; Christman, Sperry & Adler 2009). Such explanations, however, remain controversial for conifers and across conifer species, and correlations between tracheid diameter and cavitation vulnerability were attributed to the variation in the thickness-span ratio, that is, investments to avoid cell wall implosion (Pittermann *et al.* 2006b).In our analysis, we show, however, that the thickness-span ratio did not play such a role in explaining cavitation vulnerability across branches for different populations of a single pine species.

The lack of any trend in cell wall thickness effects on cavitation vulnerability does not correspond with the negative effects observed for angiosperm trees (Cochard *et al.* 2008; Fichot *et al.* 2010), but maybe this is not surprising given the different pit mechanisms preventing cavitation in angiosperms and conifers (Delzon *et al.* 2010). More surprising is the lack of any effect of wood density (after controlling for tracheid hydraulic diameter) or thickness-span ratio on cavitation vulnerability. The pines thus did not tune cell wall/lumen relations to prevent implosion, despite the fact that some of the most water-stressed sites for Scots pines were included [e.g. Salgesch in Switzerland (Zweifel, Steppe & Sterck 2007) and Prades in Spain (Martinez-Vilalta & Pinol 2002)]. It seems plausible that the required cell properties to prevent implosion are similar amongst branches, because pines maintain similar minimum leaf water potentials by stomatal regulation (Zweifel, Steppe & Sterck 2007) under different climates (Martínez-Vilalta *et al.* 2009).

The positive effects of tracheid hydraulic diameter on specific conductivity and cavitation vulnerability did not result in a clear trade-off between specific conductivity and cavitation vulnerability across branches (Fig. 4a). Our mixed models suggested that such emergent trade-offs are confounded by random site effects on cavitation vulnerability or any possible confounding factor inherent to the natural set up of our study. Yet, in a controlled experiment, there was also no support for any trade-off between cavitation vulnerability and specific conductivity across genotypes of a Populus hybrid (Fichot *et al.* 2010). Interspecific relationships between cavitation vulnerability and specific conductivity suggest trade-offs across conifers (Pittermann *et al.* 2006a,b) and angiosperms (Markesteijn *et al.* 2011), but corrected for phylogenetic dependencies across species such relationships may disappear (Maherali, Pockman & Jackson 2004). In our intraspecific study on pines, we observed that the effects of tracheid hydraulic diameter potentially cause a trade-off between specific conductivity and cavitation vulnerability (Figs 4a and 5), but the emergent bivariate correlation was nevertheless weak and not statistically significant.

We initially hypothesized that an emergent growth – cavitation vulnerability trade-off would result from the coupled effects of tracheid hydraulic diameter on cavitation vulnerability and specific conductivity, and from the effects of specific conductivity and cavitation vulnerability on growth (Fig. 2b). However, there was no direct effect of specific conductivity on growth (Table 4), implying that the expected positive influence of specific conductivity on stomatal conductance and photosynthesis is negligible. The specific conductivity correlated positively with the leaf-specific conductivity (K_l,

Table 5. The statistics for the alternative path models as shown in Fig. 2

Path model	Line style*	Exclusive paths	χ^2	df	P	AIC
1	Dashed	$(t/b)^2 \rightarrow \psi_{50}$	12·2	4	0·016	44·2
2	Dashed	$d_w \rightarrow \psi_{50}$	5·9	4	0·205	37·9
3	Dotted	$t \rightarrow \psi_{50}$	11·0	4	0·027	43·0
4	Dot-dashed	$D_h \rightarrow \psi_{50}$	4·1	4	0·396	36·0
6	Dot-dashed	$A_l : A_s \rightarrow D_h \rightarrow K_S$	3·0	3	0·385	37·0
6	Dotted	$A_l : A_s \rightarrow K_S$	25·7	3	< 0·001	59·6
7	Double-dot-dashed	$A_l : A_s \rightarrow D_h \rightarrow \psi_{50}$	3·1	3	0·380	37·1
8	Dashed	$A : A_s \rightarrow \psi_{50}$	23·5	3	< 0·001	57·5

The procedure was to compare model 1–4 and the models 5–8, and the best fitting model is shown in Fig 4a,b, respectively.

*Line styles refer to Fig. 2a for the path models 1–4, and to Fig. 2b for the path models 5–8.

$r = 0{\cdot}60$, Table 2) and partially compensated the negative impact of leaf area to sapwood area ratio on leaf-specific conductivity ($r = -0{\cdot}61$, Table 2). While the leaf-specific conductivity may therefore show more overlap across different sites than specific conductivity or leaf area to sapwood area ratio (see Fig. 3b), it nevertheless correlated positively with climate dryness (Martínez-Vilalta *et al.* 2009). The apparent trade-off between cavitation vulnerability and growth (Table 4) was caused by the positive effects of the leaf area to sapwood area ratio on both growth and tracheid hydraulic diameter, which in turn affected the cavitation vulnerability (Fig. 4). If negative effects of cavitation vulnerability on growth would still occur during dry periods, they are inferior to the (coupled) positive effects of leaf area to sapwood ratio on growth.

While new, the model is consistent with previous studies on pines. It, for example, accounts for a central role of leaf area to sapwood area ratio in the functional trait network and the association between leaf area to sapwood area ratio and specific conductivity in particular (Martínez-Vilalta *et al.* 2009), and it couples the leaf area to sapwood area ratio and tracheid diameter with growth (e.g. Sterck *et al.* 2008). It is also consistent with studies that speculate that leaf area, rather than cavitation vulnerability or specific conductivity, drives the variation in growth (Fichot *et al.* 2010). Most importantly, however, our path model analysis shows that considering functional trait networks helps to distinguish between the correlative and the functional nature of intraspecific trait correlations and the role of such traits in explaining proxies for fitness such as growth.

Conclusions

The pines of our study decreased their leaf area to sapwood area ratio in response to climate dryness, but maintained relatively constant minimum leaf water potentials across sites (Martínez-Vilalta *et al.* 2009). We showed how the variation in leaf area to sapwood area ratio and the stomatal regulation maintaining comparable hydraulic conditions cascaded down to changes in anatomical traits, hydraulic traits and growth. The negative impact of a lower leaf area to sapwood area ratio on tracheid hydraulic diameter and growth is consistent with the observed trait correlations with stand development and thus with a tendency to avoid negative leaf water potentials as tree size increases (Martínez-Vilalta *et al.* 2009). These impacts were, however, not fully consistent with trait correlations with climate dryness (Martínez-Vilalta *et al.* 2009), possibly owing to the small replication across sites ($N = 12$). Moreover, it may reflect that branches coordinate their traits in a functional, integrative manner in response to a multitude of factors (e.g. tree age, height, branch position, soil or climate), which were obviously confounded across the 12 selected sites. We disentangled functional relationships from correlative trends by analysing functional trait networks with path analysis. While it is too early to speculate about the generality of such functional trait networks for conifers, or maybe isohydric conifers, we advocate using integrated multi-trait approaches rather than bivariate relationships for understanding the responses in anatomical and hydraulic functional trait responses and their consequences for growth in branch, or whole plants.

Our approach suggests a central role for the tracheid hydraulic diameter and leaf area to sapwood area ratio in explaining the variation in cavitation vulnerability and specific conductivity, and emergent trade-offs between anatomical and hydraulic traits and growth. We show that many functional trait relationships differed from those relationships observed across species. Possibly, the strong stomatal regulation and nearly constancy of minimum leaf water potentials (Zweifel, Steppe & Sterck 2007; Martínez-Vilalta *et al.* 2009) amongst pine branches create a different playground for adaptive responses in functional traits than across species, which typically vary considerably in stomatal regulation (Maherali, Pockman & Jackson 2004). Our results also differ from the results of some studies reporting intraspecific patterns, but these were on angiosperms which differ from conifers in wood anatomical and leaf physiological mechanisms. New studies are needed to show how specific or general the proposed functional trait network is, and how it should include other key traits such as, for example, pit properties.

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