



# Relationship between foliar $\delta^{13}\text{C}$ and sapwood area indicates different water use patterns across 236 *Salix* genotypes

Friderike Beyer<sup>1,2</sup> · Ortrud Jäck<sup>1</sup> · Stefano Manzoni<sup>3,4</sup> · Martin Weih<sup>1</sup>

Received: 13 February 2018 / Accepted: 24 July 2018 / Published online: 3 August 2018  
© The Author(s) 2018

## Abstract

**Key message** The relationship between sapwood area and foliar  $\delta^{13}\text{C}$  values varies among 236 *Salix* genotypes, indicating different water use patterns across these genotypes.

**Abstract** The relationship between leaf  $\delta^{13}\text{C}$  and plant size (represented by e.g. total leaf area) has been used to analyze different water use patterns of plants. However, the total leaf area (TLA) is difficult to assess in trees. Our aims were to (i) identify a feasible predictor for TLA; (ii) estimate the effects of TLA on leaf-level  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values; and (iii) evaluate whether the relationship between leaf-level  $\delta^{13}\text{C}$  and a TLA proxy can be used to discriminate between different water use patterns. Various leaf and shoot traits of up to 236 *Salix* genotypes field-grown in Sweden and Italy were assessed and analyzed. Accumulated shoot diameter and sapwood area (SA) calculated from it were the best predictors for TLA. The SA was significantly correlated with foliar  $\delta^{13}\text{C}$ , but not  $\delta^{18}\text{O}$  values in some genotypes. The effects of SA on foliar  $\delta^{13}\text{C}$  values varied significantly among genotypes, and the foliar  $\delta^{13}\text{C}$ –SA relationship could be used to discriminate between different water use patterns across 236 *Salix* genotypes. Our results demonstrate a great variability of water use patterns across taxonomically closely related plants, and may also have implications for *Salix* pre-breeding and selection for different drought conditions.

**Keywords** Breeding for climate change · Drought · Isotopes · Water use efficiency · Intrinsic water use efficiency · Stomatal conductance

---

Communicated by Arthur Gessler.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00468-018-1747-3>) contains supplementary material, which is available to authorized users.

---

✉ Friderike Beyer  
friderike.beyer@waldbau.uni-freiburg.de

- <sup>1</sup> Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Ulls väg 16, P.O. Box 7043, 75007 Uppsala, Sweden
- <sup>2</sup> Chair of Silviculture, Faculty of Environment and Natural Resources, Albert-Ludwigs-University Freiburg, Freiburg, Germany
- <sup>3</sup> Department of Physical Geography, Stockholm University, Stockholm, Sweden
- <sup>4</sup> Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden

## Introduction

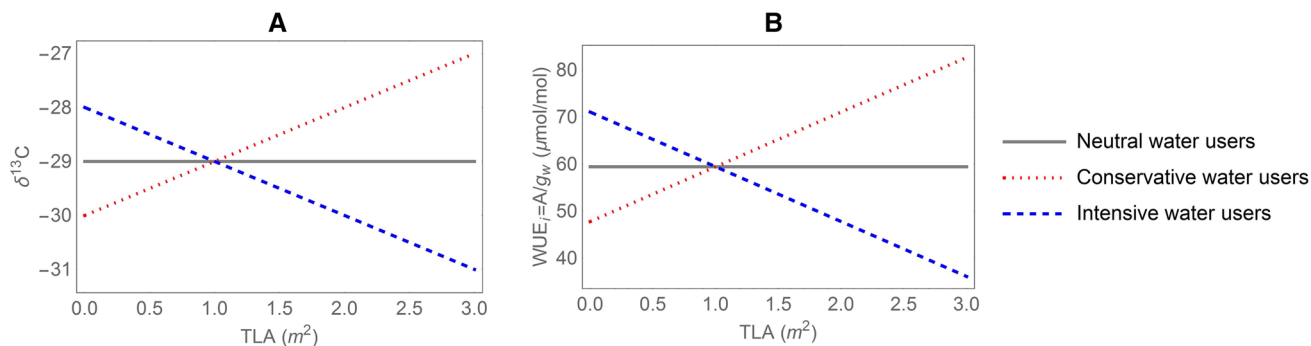
Climate change predictions call for a detailed analysis of drought induced lowered productivity and physiological adaptation strategies for crops and trees (Chaves et al. 2003). Plants can respond to limited water supply through structural and/or physiological adaptation. In most plants, a first reaction to drought is a reduced stomatal conductance, resulting in lower intercellular  $\text{CO}_2$  concentration, relatively higher leaf  $^{13}\text{C}/^{12}\text{C}$  ratio, and enrichment of  $^{18}\text{O}$  (Barbour et al. 2002). Stable isotopes of plant material are thus used to analyze the physiological performance of a plant or to a-posteriori determine the environment that a plant had been experienced during growth (Dawson et al. 2002; McDowell et al. 2008, 2011). Moreover, the carbon isotope ratio ( $\delta^{13}\text{C} = ^{13}\text{C}/^{12}\text{C}$  ratio normalized by a standard) is related to the leaf water use efficiency (WUE = net assimilation rate/transpiration rate)—a key trait when comparing adaptations of different species and genotypes to water shortage, and linking carbon and water cycles (Impa et al. 2005; Dillen et al. 2008; Brien et al. 2017). Therefore, rapid and

high-throughput analyses of WUE would benefit not only crop breeding towards resource use efficient genotypes, but also ecologists in understanding plant carbon–water relationships (Stewart et al. 1995; Condon et al. 2002; Keitel et al. 2003; Barbour et al. 2011).

Whole-plant water-use efficiency is the ratio of biomass produced to water used during a certain time period. Thus, WUE can be analyzed by the measurement of biomass production and water uptake by the plant. However, this method is only feasible in greenhouse, container or lysimeter experiments, where measurements of water input and loss can be obtained accurately. At larger scales, combined water vapor and CO<sub>2</sub> fluxes can be estimated with eddy covariance methods (Beer et al. 2009), but identifying individual plant contributions to those fluxes can be difficult. Another possibility to gain understanding about the water-use mechanisms of a plant is to consider leaf-level WUE based on the carbon-isotope ratio, which is related to the ratio of assimilation rate,  $A$ , and stomatal conductance to water vapour,  $g_w$ , referred to as intrinsic WUE,  $A/g_w = WUE_i$  (see Appendix, Farquhar et al. 1989). Specifically, the leaf  $\delta^{13}C$  becomes less negative (i.e., a relative increase in  $^{13}C/^{12}C$ ) as the  $A/g_w$  ratio increases, indicating more efficient C fixation for a given water loss (i.e., higher WUE for a given vapor pressure deficit). Therefore, trends in  $A/g_w$  with plant size (in terms of the above-ground biomass produced) and leaf area are expected to impact leaf  $\delta^{13}C$  (McDowell et al. 2011; Brienen et al. 2017). These relations between plant size and leaf-level traits may be driven by changing light environment as the plant grows, but in an even-aged stand with relatively uniform light availability they allow us to formulate alternative hypotheses on whole-plant water use based on trends in leaf  $\delta^{13}C$ . In many fast-growing trees, the total leaf area or the closely related leaf area index has been previously found to be a major determinant of plant size in terms of above-ground biomass production, especially when the assessments are made within groups of plants of

similar developmental stage (Barigah et al. 1994; Pellis et al. 2004; Weih and Nordh 2005). We therefore argue that the relationships between leaf-level isotope data and plant size can be explored by analyzing the relationships between the leaf-level isotope data and total leaf area (TLA). However, the TLA of trees cannot be easily measured in the field, but is functionally linked to several shoot and/or leaf characteristics that can be more easily assessed even in field-grown trees. Thus, using reliable predictor(s) for TLA from characteristics readily measurable in the field should considerably facilitate the identification of different water use patterns based on the analysis of the relationships between leaf-level isotope data and plant size or TLA.

In Fig. 1, three strategies of water use as plant size or TLA increases are illustrated, based on a simple model of plant water use described in Appendix A (trends in WUE and  $WUE_i$  are effectively the same as vapor pressure deficit was similar across genotypes in our setup). These strategies are defined based on the assumption that photosynthetic rates per unit leaf area do not co-vary with plant size or TLA (this assumption is relaxed below). (i) Intensive water users are able to access larger soil water stores as they grow because of deeper roots; as a result, they reduce their dependency on transpiration control by stomatal regulation as they grow larger and can keep stomata open. Thus, leaf  $\delta^{13}C$  decreases (WUE decreases) with increasing plant size or TLA (dashed blue lines in Fig. 1). (ii) In contrast, in conservative water users, larger plants with large TLA, but constrained access to water, close their stomata during dry periods to reduce water losses, consistent with isohydric regulation (Tardieu and Simonneau 1998); as a result, leaf  $\delta^{13}C$  increases (WUE increases) as size increases (dotted red lines in Fig. 1). (iii) Neutral water users can both access deep soil water and control stomatal conductance; as a result they do not change leaf  $\delta^{13}C$  (WUE) as plant size or TLA increases (solid lines in Fig. 1).



**Fig. 1** Theoretical relations between plant size (total leaf area, TLA) and leaf-level gas exchanges and C isotopic signature. **A**  $\delta^{13}C$  as a function of TLA. **B** Ratio of net assimilation rate to stomatal con-

ductance to H<sub>2</sub>O ( $WUE_i = A/g_w$ ) as a function of TLA. Different line styles refer to alternative water use patterns that affect the  $\delta^{13}C$  vs. TLA relation in A. See details in “Appendix”

It is also possible that trends in WUE are partly driven by changes in  $A$  as trees grow, because a stronger C sink in young growing trees (e.g. Hayat et al. 2017) could cause an increase in photosynthetic capacity and hence  $A$  with size or TLA. If this is the case, genotypes classified as conservative would not necessarily regulate  $g_w$  with increasing size or TLA, but simply maintain water losses while increasing  $A$  with the result that WUE (leaf  $\delta^{13}\text{C}$ ) might increase with leaf area because of trends in  $A$  rather than in  $g_w$ . Following this rationale, in intensive users, the decrease in WUE with TLA would be attributed to a simultaneous increase  $A$  and a stronger increase in stomatal conductance with TLA compared to a scenario with constant  $A$ . Therefore, if  $A$  co-varies with tree size or TLA, it becomes more difficult to classify conservative water users (because increasing WUE could be due solely to increasing  $A$ ), whereas the classification of intensive water users would not change (because decreasing WUE would still be caused by increasing  $g_w$ ).

The possibility of contrasting trends in WUE with tree size in terms of total leaf area raises two related questions that partly frame this contribution:

- Is WUE increasing or decreasing as total leaf area changes, respectively indicating a more conservative or more intensive water use strategy?
- Are trends in WUE with total leaf area consistent or divergent across the taxonomically closely related genotypes of a fast-growing tree?

These questions will be addressed by exploring the relationships between leaf  $\delta^{13}\text{C}$  and TLA across a large number of fast-growing willow genotypes.

The oxygen-isotope ratio of leaves is indicative of the stomatal conductance (Dongmann et al. 1974). The lighter  $^{16}\text{O}$  isotopes diffuse faster during evaporation and transpiration than  $^{18}\text{O}$ , leaving leaf water enriched in  $^{18}\text{O}$  and correspondingly more of the heavy isotope in the biomass. The concentration of  $^{18}\text{O}$  isotope depends also on the origin of the water, but no significant isotope fractionation is detectable during plants' uptake of water by roots (Dawson and Ehleringer 1991; Barbour 2007). Leaf  $\delta^{18}\text{O}$  was shown to correlate negatively with stomatal conductance (Barbour and Farquhar 2000; Barbour et al. 2000; Farquhar et al. 2007) and can therefore be used to determine whether differences between plants in  $\delta^{13}\text{C}$  and the ratio of  $\text{CO}_2$  mole fraction at sites of carboxylation ( $C_i$ ) to  $\text{CO}_2$  mole fraction in ambient air ( $C_a$ ) ( $C_i/C_a$ ) are caused by differences in photosynthetic capacity or in stomatal conductance (Farquhar et al. 1989; Scheidegger et al. 2000; Barbour et al. 2002; Ripullone et al. 2009; Barnard et al. 2012). Following the rationale outlined above, foliar  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  should in general be positively related, indicating a major effect of  $g_w$  on foliar  $\delta^{13}\text{C}$ , with one potential

exception for the conservative types, that might express a relatively larger impact of  $A$  on foliar  $\delta^{13}\text{C}$  in a scenario with higher  $A$  with TLA. Using leaf  $\delta^{18}\text{O}$  in addition to  $\delta^{13}\text{C}$  thus allow addressing a third question:

- Is the relative importance of stomatal conductance vs. photosynthetic capacity on foliar  $\delta^{13}\text{C}$  modulated by the total leaf area and variable across the taxonomically closely related genotypes of a fast-growing tree?

Using short rotation coppice with willows (*Salix* spp.) as model system, we first searched for an appropriate predictor of total leaf area (TLA) that could be used for characterizing the TLA of field-grown willow genotypes; and then analyzed the effect of TLA on foliar  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  to identify different water use patterns of taxonomically closely related plant material. In terms of plant functional types, willows are considered belonging to the group of early successional plants frequently colonizing newly opened habitats (Kuzovkina et al. 2008). These early-successional plants tend to be more plastic than late-successional species (Strauss-Debenedetti and Bazzaz 1991; Muth and Bazzaz 2003; Longuetaud et al. 2013), suggesting a high plasticity in terms of growth and resource use including the strategies for water use (e.g. Fig. 1). Indeed, great genotypic variation in intrinsic water-use efficiency, high genetic-based plasticity in biomass traits, and high potential to cope with dry environments has been shown within the genera *Populus* and *Salix* (Rönnerberg-Wästljung et al. 2005; Weih et al. 2006; Schreiber et al. 2011; Fichot et al. 2011; Bonosi et al. 2013; Broeckx et al. 2014; Berlin et al. 2017). Apart from improving our understanding of plant water-use mechanisms along the questions outlined above, the appropriate evaluation of genotypic variation in foliar  $\delta^{13}\text{C}$  data, separating the effect of total leaf area from genotypic variation in leaf physiology, would be helpful in a breeding context specifically for willows, as these are considered a promising alternative source of biomass for bio-energy (Karp and Shield 2008).

The overall aims of this study were to (i) identify a feasible predictor for total leaf area (TLA) based on the assessment of simple shoot and/or leaf characteristics; (ii) estimate the effects of a proxy of TLA on leaf-level  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values; and (iii) evaluate whether the relationship between leaf-level  $\delta^{13}\text{C}$  and an appropriate TLA proxy can be used to discriminate between different water-use strategies and across a set of 236 *Salix* genotypes. To address these aims, we first assessed the TLA together with various leaf and shoot traits in 21 *Salix* trees field-grown in Sweden and Italy; from these data we identified the best predictors for TLA, which we then related to leaf-level isotopic data assessed across 236 *Salix* genotypes field-grown in Italy and differing in TLA proxies.

## Materials and methods

### Plant material, field sites and experimental design

The plant material consisted of a mapping population of 472 genotypes, a  $F_1$  progeny from the cross of the female ‘78183’ (diploid, *Salix viminalis* L.) and the hybrid male ‘Björn’ (diploid, *Salix viminalis* L. x *Salix schwerinii* E. Wolf) planted in Sweden and Italy (see Berlin et al. 2010 for a description of the mapping population). The genotype ‘78183’ originates from southern Sweden, whereas the *S.*

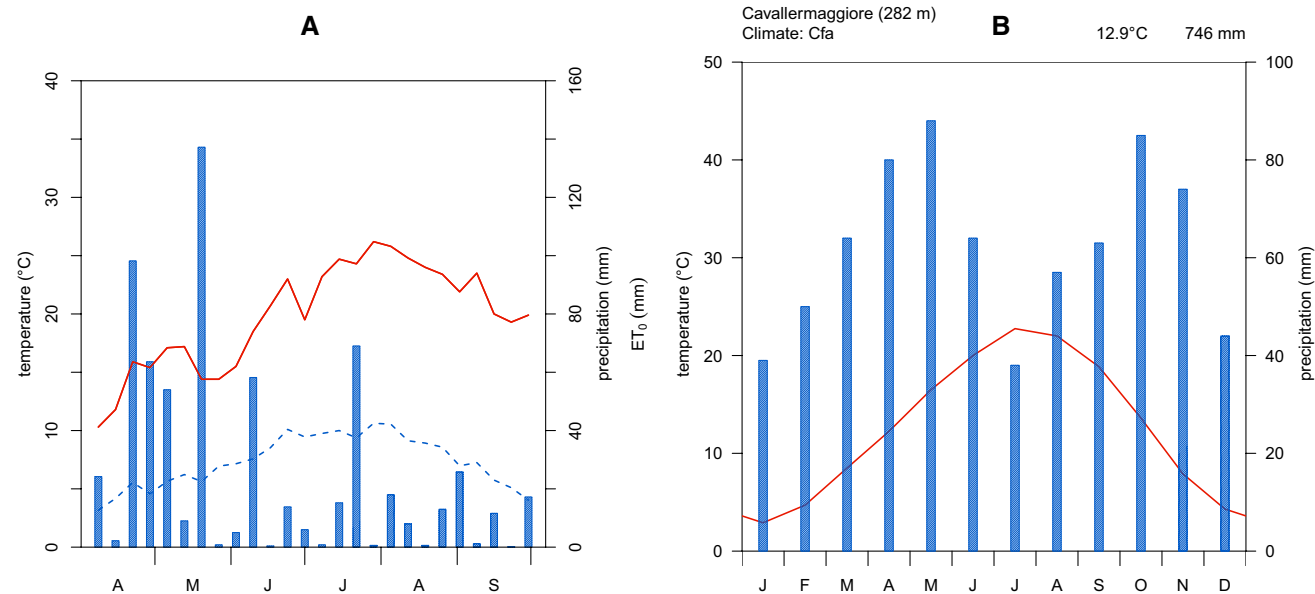
*schwerinii* parent of ‘Björn’ (79069) originates from Siberia and the *S. viminalis* parent of ‘Björn’ is a cross between the female clone ‘78195’ from southern Sweden and the male clone ‘78707’ from western Sweden.

The field experiment in Sweden was established in Pustnäs, close to Uppsala (59°48’12”N, 17°40’19”E, 18 m a.s.l.) on clayey soil near the river Fyrisån. The climate is cool-temperate with an annual mean air temperature of 5.7 °C and an annual precipitation of 551 mm (Table 1). The field trial in Sweden was fertilized with 60–80 kg/ha NPK in June 2009, 2010, 2012 and 2013. Cuttings of the mapping population of 472 different genotypes replicated sixfold were planted as a common garden experiment in a randomized block design in 2008. The plantation was harvested in the winter 2010/2011 and was cut back 2011/2012 to deliver cuttings for the experiment in Italy and to synchronize the starting date of growth in Sweden and Italy. The Swedish site was not irrigated.

The experiment in Italy was situated close to Cavallermaggiore (44°42’34”N; 7°40’42”E, 281 m a.s.l.), 40 km south of Turin. Cavallermaggiore has an average temperature of 12.9 °C and a yearly precipitation of 746 mm and is classified as humid subtropical climate (Köppen–Geiger climate classification: Cfa; Fig. 2B). Wet and dry periods alternated repeatedly during the experimental season 2013 (Fig. 2A). The cuttings from Sweden were planted in Italy during spring 2012, with the same design as in Sweden but doubled in a non-irrigated and irrigated field site.

**Table 1** Description of the experimental sites Pustnäs (Sweden) and Cavallermaggiore (Italy)

Site	Pustnäs/Uppsala/Sweden	Cavallermaggiore/Piemonte/Italy
Geographical localization		
Latitude	59°48’12”N	44°42’34”N
Longitude	17°40’19”E	7°40’42”E
Altitude (m a.s.l.)	18	282
Climate		
Mean annual temperature (°C)	5.7	12.9
Annual precipitation (mm)	551	746
Soil characteristics		
Nitrogen (g kg <sup>-1</sup> )	1.2	1.2
C <sub>org</sub> (g kg <sup>-1</sup> )	11.0	10.0



**Fig. 2** **A** Weather conditions at a weekly resolution during the experimental season 2013 in Cavallermaggiore, Italy. Average temperature (red curve), reference crop evapotranspiration rate (dashed blue curve), and weekly precipitation sums (bars) are displayed. Data

are based on interpolation [data source: MARS-AGRI4CAST, 2017 (European Union)]. **B** Average temperature (red curve) and monthly precipitation sums (bars) for Cavallermaggiore, based on the 30-year period 1982–2012 (data-source: <http://www.climate-data.org>)

### Prediction of total leaf area

Assessment of total leaf area (TLA) of mature trees is labor intensive and a reliable predictor of TLA facilitates evaluation of the relationships to growth and physiological traits. To identify a reliable predictor for TLA we conducted a pilot study where seven trees were harvested in Pustnäs (Sweden) in July 2013. Altogether we harvested seven plants consisting of 84 shoots in total with a height above 1 m. The following parameters were tested: leaf areas within different shoot sections (within 20, 30, 40 and 50 cm) along the main shoot, main shoot height, main shoot diameter, main shoot crown length, number of shoots, total crown height, total height, total leaf biomass, total diameter at 1.20 m height (Table 2). Sapwood area (SA) was calculated assuming circular shoot cross-sections and that sapwood extended to the center of the shoot (a reasonable assumption for these young shoots). Specific leaf area ( $\text{m}^2 \text{g}^{-1}$ ) was analysed by assessing individual leaf areas with a flat-bed scanner (Epson Perfection V370 Photo) and the open-source software ImageJ (Yang Yu et al. 2011), and the subsequent determination of leaf dry weights after drying the leaves for 48 h at 70 °C. In September 2013, additionally 14 trees were sampled in Italy for determination of TLA and stem diameter.

### Biomass inventory and isotopic analyses

Due to time and cost restrictions, we were unable to perform isotope analyses across all the available treatments and sites. With regard to the underlying hypotheses of this study, highest priority was given to the non-irrigated site in Italy, because the dry conditions in this treatment offered

the enhanced likelihood to detect distinct patterns of water use across the various genotypes included in this study. We analyzed 236 different genotypes replicated fourfold in September. Diameters at 1.20 m height of each living shoot were measured in September 2013, integrating major parts of the growth period of the same year in the isotopic signal. Three intact leaves, one at 1, 1.5 and 2 m height each, were taken from the main shoot (i.e. the tallest shoot) of each plant and pooled. After drying the leaf samples for 48 h at 70 °C the samples were homogenized in a mixer ball mill (MM400, Retsch GmbH, Haan, Germany) at 30 Hz for 1 min, the material was analysed with a mass spectrometer (Elemental Analyser—Isotope Ratio Mass Spectrometry (EA-IRMS) at Iso-Analytical Limited, Cheshire, UK). Isotope values are specified as deviations ( $\delta$ , ‰) relative to specific standards. V-PDB (Vienna standard for fossil belemnite of the Pee Dee formation) is used for  $^{13}\text{C}/^{12}\text{C}$ , and V-SMOW (Vienna Standard Mean Oceanic Water) for  $^{18}\text{O}/^{16}\text{O}$ :

$$\delta_{\text{sample}} = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1,$$

where  $R$  is the ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{18}\text{O}/^{16}\text{O}$  in the sample or standard.

The reference material used during  $\delta^{13}\text{C}$  analysis of the plant samples was IA-R001 (wheat flour,  $\delta^{13}\text{C}_{\text{V-PDB}} = -26.43\text{‰}$ ). For quality control purposes check samples of IA-R001, IA-R005 (beet sugar,  $\delta^{13}\text{C}_{\text{V-PDB}} = -26.03\text{‰}$ ) and IA-R006 (cane sugar,  $\delta^{13}\text{C}_{\text{V-PDB}} = -11.64\text{‰}$ ) were analysed during batch analysis of the samples. IA-R001, IA-R005 and IA-R006 are calibrated against and traceable to IAEA-CH-6 (sucrose,  $\delta^{13}\text{C}_{\text{V-PDB}} = -10.43\text{‰}$ ).

**Table 2** Predictors for total leaf area per plant

	<i>n</i>	Mean	SD	Min	Max	RSE	Multiple $R^2$	$R^2_{\text{adj}}$	<i>F</i>	<i>P</i> value	<i>DF</i>
Leaf area within 20 cm shoot length ( $\text{cm}^2$ )	5	4.99	5.82	0.49	13.47	18,860	0.741	0.611	5.71	0.139 <sup>n.s.</sup>	2
Leaf area within 30 cm shoot length ( $\text{cm}^2$ )	5	2.86	3.38	0.32	7.83	35,990	0.056	-0.416	0.12	0.764 <sup>n.s.</sup>	2
Leaf area within 40 cm shoot length ( $\text{cm}^2$ )	5	12.38	13.69	0.62	32.14	8424	0.948	0.922	36.67	0.026*	2
Leaf area within 50 cm shoot length ( $\text{cm}^2$ )	5	33.84	37.03	1.62	82.47	9771	0.93	0.896	26.74	0.035*	2
Main shoot crown length (m)	7	1.57	0.49	0.78	2.20	15,290	0.779	0.706	10.59	0.047*	3
Main shoot diameter (mm)	7	13.27	5.93	3.06	22.20	22,150	0.537	0.383	3.48	0.159 <sup>n.s.</sup>	3
Main shoot height (m)	7	3.05	1.10	1.24	4.82	26,440	0.34	0.12	1.55	0.302 <sup>n.s.</sup>	3
Number of shoots	7	14.43	9.32	3.00	24.00	21,080	0.581	0.441	4.15	0.134 <sup>n.s.</sup>	3
Total crown height (m)	7	16.95	14.23	2.58	37.63	4414	0.982	0.976	160.1	<0.01**	3
Total height (m)	7	29.60	22.70	4.42	57.00	8744	0.937	0.917	44.92	<0.01**	3
Total leaf biomass (g)	7	221.91	250.03	8.82	685.40	1251	0.999	0.998	2026	<0.001***	3
Total Diameter (mm)	7	101.87	86.27	7.06	213.10	5052	0.976	0.968	121.5	<0.01**	3
Total sapwood area ( $\text{mm}^2$ )	7	922.21	904.48	13.64	2214.41	4583	0.98	0.974	148.3	<0.01**	3

Pilotstudy in Sweden with seven plants with a total of 84 shoots. Leaf area within different sections of shoot length (20–50 cm) was analysed for five plants and only on the main shoot (the thickest shoot of each plant). RSE is the residual standard error of the estimate,  $R^2_{\text{adj}}$  is the coefficient of variation, *DF* are the degrees of freedom. Significance codes: \*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ ;  $p < 0.1$ ; *n.s.* not significant. Highly significant regressions are indicated by bold fonts ( $p < 0.01$ )



The reference material used for  $\delta^{18}\text{O}$  analysis was IA-R006 (cane sugar,  $\delta^{18}\text{O}_{\text{V-SMOW}} = 35.23\text{‰}$ ). For quality control purposes test samples of IA-R006, IA-R005 (cane sugar,  $\delta^{18}\text{O}_{\text{V-SMOW}} = 30.84\text{‰}$ ), IAEA-CH-6 (sucrose,  $\delta^{18}\text{O}_{\text{V-SMOW}} = 36.4\text{‰}$ ), IAEA-C-3 (cellulose,  $\delta^{18}\text{O}_{\text{V-SMOW}} = 32.2\text{‰}$ ) and IAEA-601 (benzoic acid,  $\delta^{18}\text{O}_{\text{V-SMOW}} = 23.3\text{‰}$ ) were measured as quality control checks during analysis of each batch of the samples. IA-R006, IA-R005, IAEA-CH-6 and IAEA-C-3 were oven dried alongside the samples for 10 days directly prior to analysis to remove moisture. Because IAEA-601 does not absorb water and is liable to decompose on heating it was not oven dried along with the samples.

## Statistical analyses

The statistical analysis of the data and graphical illustrations were performed with the free software environment R (version R 3.2.1., R Core Team 2014, The R Foundation for Statistical Computing, <http://www.r-project.org>) with the packages ggplot and lsmeans. Multiple linear regression analysis was performed to analyze the relationship between sapwood area and  $\delta^{13}\text{C}$ . The regression model included the categorical variables block and genotype, the continuous variable sapwood area (covariate), and the interaction between genotype and sapwood area. Additionally, we performed a  $p$  value adjustment for multiple comparisons with false discovery rate (FDR) by Benjamini and Hochberg with an alpha-level of 0.05. An analysis of covariance (ANCOVA) was performed to test whether any effect of sapwood area on response variables (isotope data) varied between genotypes. The linear model included the categorical variables block and genotype, the continuous variable sapwood area, and the interaction between genotype and sapwood area with type I sum of squares. The same procedure was applied in the follow-up analysis separately done for each of the water use patterns that had been identified, to test the response of  $\delta^{18}\text{O}$  to genotype and sapwood area as well as the relationship between  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and genotype. In all analyses a significance level of  $\alpha = 0.05$  was chosen.

## Results

### Prediction of total leaf area

From the allometric measurements we identified four parameters for which the correlations to total leaf area (TLA) were significant (Table 2). Among them, the parameters with the best fit were total crown height ( $R^2_{\text{adj}} = 0.982$ ,  $p < 0.001$ ), total height ( $R^2_{\text{adj}} = 0.937$ ,  $p < 0.001$ ), accumulated shoot diameter ( $R^2_{\text{adj}} = 0.976$ ,  $p < 0.01$ ) and total sapwood area ( $R^2_{\text{adj}} = 0.980$ ,

$p < 0.001$ ). Total leaf biomass showed the best correlation with TLA ( $R^2_{\text{adj}} = 0.999$ ,  $p < 0.0001$ ), but is less feasible as a predictor given the intensive labor for assessing it. From a time–cost efficient point of view diameter at 1.20 m height and the sapwood area calculated from it, were regarded the most feasible parameters and used for prediction of TLA (Figure S1).

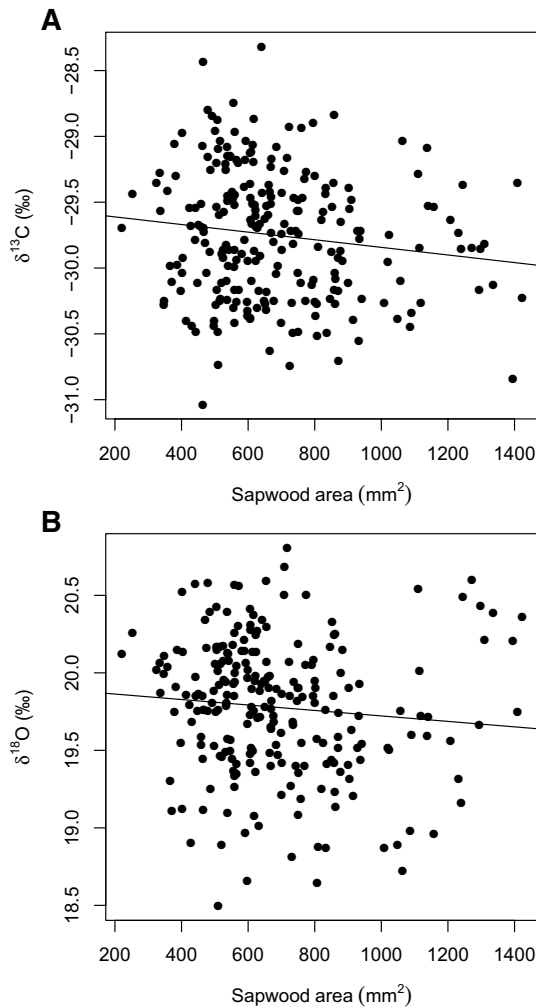
### Influence of sapwood area and genotype on isotope values

Linear regressions of foliar  $\delta^{13}\text{C}$  and sapwood area revealed significant relationships (Fig. 3A). Sapwood area and genotype strongly affected the  $\delta^{13}\text{C}$  values ( $p < 0.0001$ ; Table 3), and the effect of sapwood area (here indicative of TLA) on  $\delta^{13}\text{C}$  values varied significantly between the genotypes (interaction effect,  $p = 0.0382$ ; Table 3). No significant regression between  $\delta^{18}\text{O}$  and sapwood area was found (Fig. 3B), however the ANCOVA model showed significant influence of sapwood area ( $p < 0.0001$ ) and genotype ( $p < 0.0001$ ) as well as the interaction of those on  $\delta^{18}\text{O}$  ( $p < 0.01$ ; Table 3).

### Identification of different water use patterns

Linear regressions of foliar carbon isotope values against sapwood area (SA) across the 236 willow genotypes indicated different water use patterns (as conceptually defined in Fig. 1); i.e. neutral and intensive water users (Fig. 4A, B for specific examples). The majority of the 236 *Salix* genotypes showed no significant relationship between leaf  $\delta^{13}\text{C}$  values and SA (Table S1) and most of the slope estimates were close to zero (Fig. 4A, Figure S2a). Applying false discovery rate adjusted  $p$ -values after Benjamini and Hochberg resulted in four genotypes with a significant ( $p < 0.05$ ) negative relationship between  $\delta^{13}\text{C}$  values and SA (indicative of an intensive water use strategy).

The relationship between leaf  $\delta^{18}\text{O}$  and sapwood area was genotype-specific, both in the group of intensive and neutral water users (Table 4). Three out of four intensive water users had a significantly negative relationship (Table S3) indicating increased stomatal conductance to water vapor ( $g_w$ ) in larger plants, whereas in the group of the 232 neutral water users the majority revealed no significant relationships and only one had a significantly negative relationship. Leaf  $\delta^{13}\text{C}$  was generally positively correlated with leaf  $\delta^{18}\text{O}$  for the group of neutral water users (Table 4) with the intensive water users having a steeper slope (1.623) compared to the neutral water users (0.176) indicating increasing intrinsic WUE with decreasing  $g_w$  (Fig. 5).



**Fig. 3** Linear regressions of mean values for each of the 236 genotypes (replicated 3–4 times) between sapwood area and isotopic values of leaves. **A** Foliar  $\delta^{13}\text{C}$  as function of sapwood area at 1.20 m height ( $n=236$ ),  $R^2_{\text{adj}} = 0.01489$ ,  $p=0.0339$ , linear regression:  $y = -29.55 - 0.000289x$ . **B** Foliar  $\delta^{18}\text{O}$  as function of sapwood area at 1.20 m height ( $n=236$ ),  $R^2_{\text{adj}} = -0.004512$ ,  $p=0.152$ , linear regression:  $y = 19.89854 - 0.0001763x$

## Discussion

In this study, we screened a population of 236 taxonomically closely related *Salix* genotypes for characteristics indicative of different plant water-use patterns; i.e., the individual relationships between foliar carbon isotope values and total leaf area. Despite the close taxonomic relatedness in the plant material, we found contrasting water use patterns. Some genotypes exhibited statistically significant decrease in WUE with increasing size, suggesting increasing access to water via deeper roots (intensive users; Fig. 1). Nevertheless, the majority of genotypes were characterized as neutral, i.e. with no significant relationship between foliar carbon isotope values and sapwood area. This variability in water use patterns is in line with previous reports on the large phenotypic and genetic variability of biomass and water use characteristics in *Salix* (Rönnerberg-Wästljung et al. 2005; Weih et al. 2006; Berlin et al. 2017), which provides a promising basis for (pre-)breeding research targeting biomass willows adapted to local environments differing in water availability.

### Sapwood area as an indicator of total leaf area

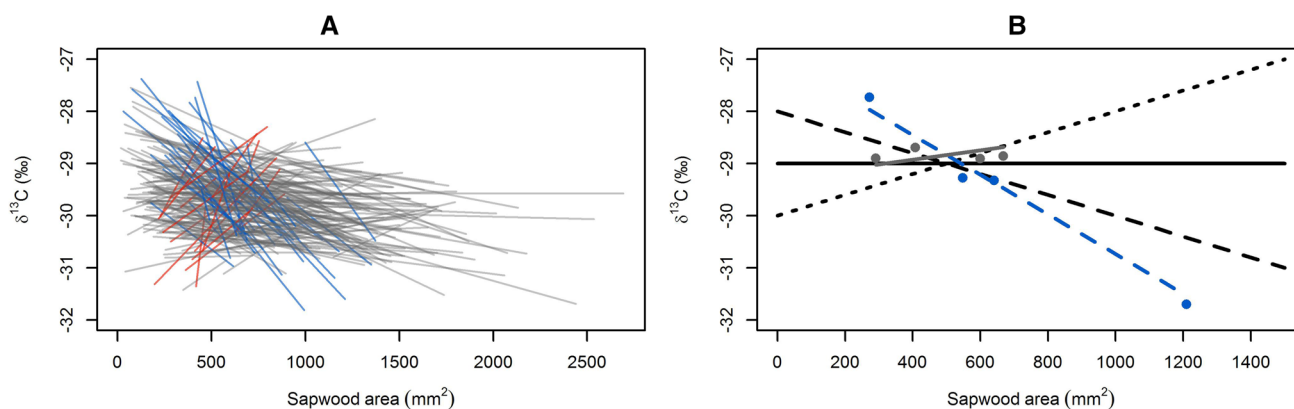
Systematic allometric relationships between sapwood area and whole-tree leaf area have been recognized long time ago mainly for single-stem trees (Huber 1928; Waring et al. 1982), and our results confirm this relationship also for *Salix* with multiple stems. The leaf area vs. sapwood area relation has a slope of 1.16 (Figure S1) similar to the theoretical value of 1 for an idealized fractal tree (Hunt and Manzonei 2015) calculated based on the allometric model by Savage et al. (2010).

The stem diameter is considered a good predictor of biomass (Annighöfer et al. 2012; Albert et al. 2014) as well as a proxy for most of the anatomical features of trees (Vertessy et al. 1995). Sapwood area is often closely correlated with the diameter at breast height (Sellin 1994; Bond-Lamberty et al. 2002), and the area of stem cross-section can be used as a proxy for sapwood area especially in trees with thin bark

**Table 3** ANCOVA table of  $F$  and  $p$  values on the effect of genotype and sapwood area and their interaction on isotopic parameters

Traits	$R^2_{\text{adj}}$	Source of variation					
		Genotype ( $DF$ 235)		Sapwood area ( $DF$ 1)		Genotype $\times$ sapwood area ( $DF$ 235)	
		$F$	$P$	$F$	$P$	$F$	$P$
$\delta^{13}\text{C}$	0.324	1.86	<0.0001***	87.96	<0.0001***	1.23	0.0382*
$\delta^{18}\text{O}$	0.292	1.57	<0.0001***	42.87	<0.0001***	1.32	0.0093**

Significance codes: \*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ .  $p < 0.1$ , *n.s.* not significant



**Fig. 4** Linear regressions of **A** foliar  $\delta^{13}\text{C}$  against sapwood area ( $\text{mm}^2$ ) separately for 236 *Salix* genotypes. Colors are indicative of the water-use strategy; grey: neutral water users, blue: intensive water users, red: conservative water users. The threshold slopes for the respective color groups were: slope  $< -0.025$ : blue,  $-0.25 \leq \text{slope} \leq 0.25$ : grey, slope  $> 0.25$ : red. Regression parameters and  $p$  values are presented in Table S1; colors do not indicate that slopes are significantly larger or smaller than zero, but only illustrate

the variability in the observed slopes. **B** Regressions between foliar  $\delta^{13}\text{C}$  (per mil) and sapwood area ( $\text{mm}^2$ ) at 1.20 m height for three *Salix* genotypes representing different water use patterns. Grey: neutral water user, blue: intensive water user (no conservative water users are shown, as no relation was significantly positive). The solid, dashed and dotted black lines represent the model outcome of the water use patterns presented in Fig. 1

**Table 4** ANCOVA table on the effect of genotype and sapwood area and their interaction on leaf  $\delta^{18}\text{O}$  and genotype  $\delta^{18}\text{O}$  on leaf  $\delta^{13}\text{C}$  within genotype groups classified as intensive and neutral water users

	Intensive water users			Neutral water users		
	$F$	$p$ adj	$R^2_{\text{adj}}$	$F$	$p$ adj	$R^2_{\text{adj}}$
$\delta^{18}\text{O}$						
Genotype	8.981	0.0299*	0.969	1.566	<0.001***	0.2813
Sapwood area	69.187	0.0011**		39.373	<0.001***	
Genotype $\times$ sapwood area	37.635	0.0022**		1.269	0.0214*	
$\delta^{13}\text{C}$						
Genotype	1.674	0.3085	0.6912	1.806	<0.001***	0.2628
$\delta^{18}\text{O}$	18.347	0.0128*		19.21	<0.001***	
Genotype $\times$ $\delta^{18}\text{O}$	0.659	0.6186		1.163	0.0986	
$\delta^{13}\text{C}$						
Genotype	1.96	0.2086	0.7363	1.699	<0.001***	0.2163
$\delta^{18}\text{O}$	21.482	0.0024**		18.071	<0.001***	

The classification was based on false discovery rate adjusted  $p$ -values after Benjamini and Hochberg ( $p$  adj. Table S1 and S2; Genotypes whose negative slope ( $d^{13}\text{C} \sim \text{Sapwood area}$ ) is significant at  $p < 0.05$  were classified as intensive water user)

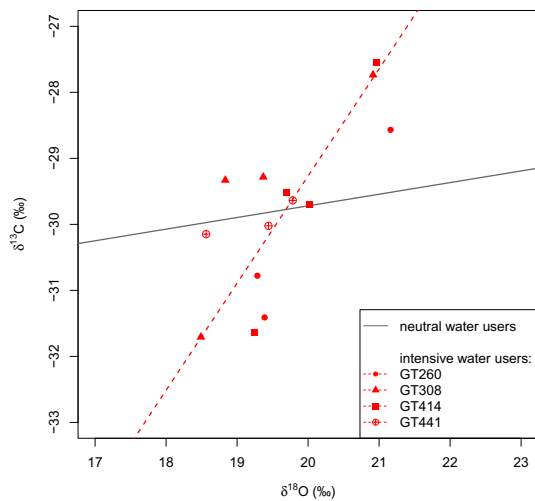
and low heartwood fraction, such as young willows. Our results confirm both accumulated stem diameter and sapwood area as reliable predictors for total leaf area in *Salix*, information necessary for the following investigation of the relationships between isotope data and total leaf area.

### Linking total leaf area to isotope values, and the influence of genotype

We found partly significant influence of sapwood area (and therefore total leaf area) on leaf carbon isotope values, but the effect of sapwood area on the isotope values varied

between the 236 genotypes. On the one hand, total leaf area is a key trait affecting whole-plant growth and yield also in *Salix* (Weih and Nordh 2005). On the other hand, leaf isotopes reflect important characteristics for plant growth and production at leaf level in relation to the amount of water consumed, i.e., of the ratio of photosynthetic over transpiration rates, integrated over the lifetime of the leaf. Many studies use foliar  $\delta^{13}\text{C}$  values to analyze intrinsic water use efficiency ( $\text{WUE}_i = A/g_w$ ) in response to drought, assuming that leaf-level  $\text{WUE}_i$  assessed by means of foliar  $\delta^{13}\text{C}$  is indicative also for whole-plant water use efficiency (Schifman et al. 2012; Toillon et al. 2013). Other studies assessed the





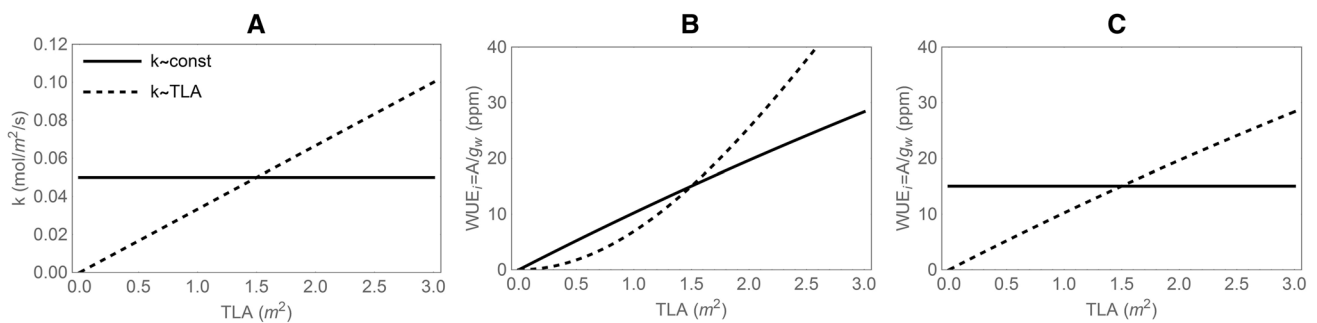
**Fig. 5** Regressions between foliar  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  for neutral water users (grey line) and intensive water users (red line and symbols). The relationship was the same for all water use efficient genotypes (Table 4). After Benjamini and Hochberg adjustment of the intercepts'  $p$  values, there was no significant genotype-effect for the neutral water users

relation between plant size and tissue  $\delta^{13}\text{C}$ , showing variable influence of total leaf area on foliar carbon isotope values. For example, Buchmann et al. (1997) assessed foliar  $\delta^{13}\text{C}$  in evergreen and deciduous forest ecosystems in semi-arid Utah, where foliar  $\delta^{13}\text{C}$  did not change with increasing leaf area index (LAI) in the tree layer. Other investigations found significant negative correlation between total leaf area and  $\delta^{13}\text{C}$  (Zhang et al. 2004; Yin et al. 2004) or between  $\delta^{13}\text{C}$  and total shoot biomass, which is in turn related to leaf area (Toillon et al. 2013). Moreover, leaf and sapwood  $\delta^{13}\text{C}$  consistently increase with tree height due to combined increased light availability (higher  $A$ ) and lower stomatal conductance (lower  $g_w$ ) in the upper parts of natural canopies (McDowell

et al. 2011). Therefore, variable relations between plant size and isotopic signature are widespread.

In the Appendix (Eq. 11), we summarized the relation between leaf-level WUE, which is related to  $\delta^{13}\text{C}$ , and several leaf and plant traits: the carboxylation capacity ( $k$ ), the soil-to-leaf hydraulic conductance ( $g_{SL}$ ), the average soil-to-leaf water potential difference ( $\Delta\psi$ ), and the sapwood area to leaf area ratio (SA/TLA). This relation provides a basis for explaining the observed variability in the plant size-isotopic signature relation. Figure 6 illustrates trends in  $\text{WUE}_i$  when leaf area is increased for fixed sapwood area (Fig. 6B) or fixed SA/TLA ratio (Fig. 6C), and when  $k$  varies with tree size according to two scenarios (constant  $k$ , or linearly increasing  $k$  with TLA, indicating higher light capture). As shown in Fig. 6B, increasing TLA for a given SA lowers stomatal conductance because the water supply to leaves is relatively lower. This in turn increases WUE in larger trees (i.e.,  $\text{WUE}_i$  is inversely proportional to SA/TLA in Eq. (11)). In this case, changing the dependence of  $k$  on TLA does not alter the general shape of  $\text{WUE}_i$ -SA relation. In contrast, keeping a stable SA/TLA ratio during growth may result in increasing of  $\text{WUE}_i$  with increasing TLA (Fig. 6C). Specifically,  $\text{WUE}_i$  is size invariant only when  $k$  is also size invariant.

These arguments hold as a first approximation only, and notably Fig. 6 does not explain why larger trees might exhibit decreasing  $\delta^{13}\text{C}$ , WUE and  $\text{WUE}_i$  with increasing total leaf area (dashed blue curves in Fig. 1). In fact, if trees experience different environments as they grow (e.g., changing water availability), it is possible that  $g_{SL}$  and  $\Delta\psi$  also vary with tree size (and TLA), but due to the confounding effect of environmental conditions. Even if the results shown in Figure A1 neglect these effects, they are accounted for in Eq. (11). For example, higher water availability as trees grow (thanks to deeper roots) might increase  $g_{SL}$ , thus decreasing  $\text{WUE}_i$  with increasing TLA, as discussed in Sect.



**Fig. 6** **A** Different assumed relations between carboxylation capacity ( $k$ ) and total leaf area (TLA);  $k$  is assumed to be constant or linearly increasing with TLA. **B**, **C** Patterns in intrinsic WUE ( $\text{WUE}_i$ ) with increasing TLA, as driven by the different  $k$ -TLA relations shown in **A**, when sapwood area is either assumed constant (**B**) or proportional to TLA (**C**). Parameters for Eq. (11) (chosen for illustration):

$c_a = 400 \mu\text{mol/mol}$ ,  $\Delta\psi = 1 \text{ MPa}$ ,  $D = 0.01 \text{ mol/mol}$ ,  $g_{SL} = 25 \text{ mol}/(\text{m}^2 \text{ s MPa})$ ,  $k = 0.05 \text{ mol}/(\text{m}^2 \text{ s})$  (baseline constant; to model changes with TLA, the baseline  $k$  value is multiplied by  $\text{TLA}/1.5$ ),  $\text{SA} = 750 \text{ mm}^2$  (baseline constant; to model changes with TLA, the SA/TLA ratio was assumed equal to  $500 \text{ mm}^2/\text{m}^2$ )

“Relationship between sapwood area and foliar carbon isotope values as indicator of water use patterns”. As our model neglects vertical variations in hydraulic traits and pressure, it is not adequate as a general model for larger trees (Couvreur et al. 2018), but it is suitable for short trees such as willows in short-rotation coppices, as in our case.

The highly variable effects of sapwood area (and total leaf area) on leaf carbon isotope values (Fig. 4) affect the interpretation of leaf-level isotopic signatures for evaluating plant-level WUE. The general problem of extrapolating  $\delta^{13}\text{C}$  values from a leaf (or a subsample of leaves) to a whole tree has been recognized previously, but we are not aware of any approach to systematically analyze the effects of changed leaf area on leaf isotope values. Such an analysis appears relevant for example in the context of plant breeding, because any genotypic variation in leaf area or drought-induced leaf-area changes could potentially mask the genotypic pattern in foliar  $\delta^{13}\text{C}$  values and affect its interpretation. Genetic variation in both total leaf area and WUE (*sensu* foliar isotope values) has been found in many plants including willow (Rönnerberg-Wästljung et al. 2005; Weih et al. 2006; Pucholt et al. 2015). Our results indicate that breeding for WUE, when assessed solely through leaf  $\delta^{13}\text{C}$ , can be misleading if results are not supported by appropriate data upscaling to the whole plant. Also a study using tree ring carbon isotopes came to the conclusion that tree height (likely a proxy of total leaf area in that study) and developmental stage strongly affected the  $WUE_i$  (McDowell et al. 2011). Thus, the combined evidence from the literature and our results indicates that single leaf measurements are not sufficient when the whole tree WUE is of interest, as for example the effects of a proxy of total leaf area (i.e., sapwood area) on leaf-level  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were considerable and interacted with genotype in this study.

Isotopic analyses with  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  can give valuable time-integrated information (either for vegetation period, when analyzing leaves or over several years when comparing year rings) on the state of trees in relation to water use. In this context, the dual isotope analysis is highly useful to assess differences in plant growth as well as total transpiration and supplies information about time-integrated photosynthetic and evaporative performance (Cabrera-Bosquet et al. 2011). Regarding foliar  $\delta^{18}\text{O}$ , the literature is scarce, and both close and no correlations between isotope ratios of  $\delta^{13}\text{C}$  and of  $\delta^{18}\text{O}$  to environmental parameters were found (Barbour et al. 2002).

### Relationship between sapwood area and foliar carbon isotope values as indicator of water use patterns

Leaves maintaining open stomata can achieve high photosynthetic rates for a given photosynthetic capacity. However,

high stomatal conductance causes large water losses via transpiration (for a set vapor pressure deficit). Because the relation between photosynthetic rate and stomatal conductance is concave downward, the leaf-level water use efficiency decreases as stomatal conductance increases (Farquhar and Sharkey 1982; Medrano et al. 2009). Therefore, patterns in WUE—as estimated from  $\delta^{13}\text{C}$ —can provide insights on trends in stomatal conductance and thus water use patterns by plants growing in the same environment (Dawson et al. 2002), as is the case for the *Salix* genotypes considered here. Specifically, we asked how tree size (in terms of TLA) may affect water use patterns in closely-related genotypes, and whether different genotypes exhibit contrasting patterns.

As argued in Fig. 1, increasing WUE with TLA (or sapwood area) indicates increasingly conservative water use, whereas decreasing WUE with plant size suggests larger water consumption per unit carbon fixed via photosynthesis (intensive water use). Both  $A$  and  $g_w$  can act as drivers of WUE. As Johnsen et al. (1999) described based on a basic mechanistic models of carbon discrimination (Farquhar et al. 1989),  $\Delta^{13}\text{C}$  and plant size should be negatively correlated (i.e., positively correlated for  $\delta^{13}\text{C}$ ) if the source of variation is photosynthetic capacity and positively (i.e., negatively for  $\delta^{13}\text{C}$ ), if the source of variation is stomatal conductance (Johnsen et al. 1999). In their study with full-sib families of *Picea mariana* they identified photosynthetic capacity as major source of variation of  $\Delta^{13}\text{C}$ . The tested *Salix* population showed high variation in the relationship between leaf  $\delta^{13}\text{C}$  and sapwood area. Thus, we found a great variability of water use patterns and could identify a few genotypes that differed from the rest of the population by having a significantly negative relationship (intensive water users). Different from the results of Johnsen et al. (1999) but in accordance with their hypothesis, we can conclude from our data that the negative correlation between  $\delta^{13}\text{C}$  and sapwood area of intensive water users among the tested *Salix* genotypes is mainly driven by stomatal conductance. The strong positive relationship between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  indicates that  $g_w$  is driving the trends in WUE ( $\delta^{13}\text{C}$ ) of these genotypes to a larger extent compared to the neutral water users. The majority of the intensive water users had a negative correlation between  $\delta^{18}\text{O}$  and sapwood area, which is indicative of higher transpiration (increased  $g_w$ ) as plant size increases. In contrast, the majority of the neutral water users had no significant relationship between  $\delta^{18}\text{O}$  and sapwood area with only one genotype having a negative relationship. In combination with the lower slope of the  $\delta^{13}\text{C}$  -  $\delta^{18}\text{O}$  relationship, these results indicate a relatively smaller effect of stomatal conductance on WUE.

The intensive and less efficient use of water caused by relatively higher  $g_w$  may be explained by considering the drivers of  $g_w$ . The conceptual model developed in Appendix A links WUE and  $\delta^{13}\text{C}$  values to  $g_w$  via plant hydraulic traits,

measures of plant size (SA and TLA), photosynthetic capacity, and environmental conditions (Eq. 11). The obtained relation elucidates how both environmental and plant features can mediate changes in  $g_w$ , thereby explaining trends in WUE with leaf area. Among the possible explanations, we argue that larger plants also grow deeper or denser roots. This argument is supported by the observation that rooting depth (assuming bedrock or hard pans are too deep to interfere, as is the case in the deep alluvial deposit of our study site) scales linearly with canopy height (Kempes et al. 2011), which in turn scales as leaf area to the power three (West et al. 1999). This means that rooting depth increases monotonically with leaf area, supporting our claim that trees with higher leaf area allow accessing water in an increasingly large soil volume. Access to more soil water increases the hydraulic conductance of the whole soil-to-leaf hydraulic pathway, thereby decreasing WUE (Eq. 11). Therefore, intensive water use may be fueled by deep water during the driest months of the growing season.

$WUE_i$  has been identified as a complex multi-trait influenced by leaf structure, biochemistry and the leaf's diffusive properties (Gago et al. 2014). Our findings suggest that the evaluation of the relationship between leaf-level  $\delta^{13}C$ ,  $\delta^{18}O$  and an appropriate leaf area proxy can be used to discriminate between different water-use strategies across different species and genotypes. The genetic diversity of our plant material resulted in a high phenotypic variability during drought for the whole stand, which is from a plant ecology perspective an essential survival strategy for the population (Lamy et al. 2013; Franks et al. 2014), and indicates opportunities for plant breeding with the possible selection for different water use patterns using the relationship between leaf-level  $\delta^{13}C$ ,  $\delta^{18}O$ , and an appropriate leaf area proxy.

**Author contribution statement** FB and MW planned and designed the experiment. FB performed the sampling and assessments and conducted the field and lab work. FB and OJ analysed the data. SM established the theoretical model. FB, OJ, SM and MW jointly wrote the manuscript.

**Acknowledgements** We would like to thank Nils-Erik Nordh, Richard Childs and Vincenz Reith for the help during field work and Simona Ferrutta, Roberto Robetti and co-workers from ALLASIA PLANT in Cavallermaggiore, Italy for the establishment and maintenance of the experiment. Giulia Vico gave helpful comments for the analysis. Funding was gratefully received from the Swedish Energy Agency (30599-3). SM and MW acknowledge partial support from the Swedish Research Council Formas (2016-00998).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## Appendix

In this section we summarize a simple leaf photosynthesis and transpiration model, which is used to frame our hypotheses on the relations between plant size (in terms of leaf and sapwood area) and leaf  $\delta^{13}C$  (Fig. 1). The model rationale follows previous works, where assumptions and limitations are discussed in depth (Lloyd and Farquhar 1994; Martinez-Vilalta et al. 2014; Brienen et al. 2017, and references therein). First, intrinsic water use efficiency ( $WUE_i$ ) is calculated as a function of leaf  $\delta^{13}C$  to explain how measured patterns in leaf  $\delta^{13}C$  can be interpreted as changes in water use patterns. Second,  $WUE_i$  is formulated as a function of leaf and sapwood areas, tissue-level hydraulic traits, and photosynthetic capacity to elucidate how size-dependent trait values may affect the plant water use strategy (Fig. 6).

## Linking C isotopic discrimination and water use efficiency

The transpiration rate per unit leaf area,  $E$ , is modeled as a diffusion process assuming that the leaves are well coupled to the atmosphere,

$$E = g_w D, \quad (1)$$

where  $g_w$  is the stomatal conductance to water vapour and  $D$  is the vapor pressure deficit expressed as molar fraction. Carbon uptake per unit leaf area can also be described as a diffusion process regulated by stomatal conductance as

$$A = g_c (c_a - c_i), \quad (2)$$

where  $g_c$  is the stomatal conductance to  $CO_2$ ,  $c_a$  is the atmospheric  $CO_2$  concentration, and  $c_i$  is the  $CO_2$  concentration in the stomatal cavity. Once inside the leaf,  $CO_2$  is fixed through photosynthesis.

Combining the definition of transpiration rate in Eqs. (1) and (2) for the net assimilation rate, we can express the leaf water use efficiency as,

$$WUE = \frac{A}{E} = \frac{c_a}{aD} \left( 1 - \frac{c_i}{c_a} \right), \quad (3)$$

where  $a = 1.6$  is the ratio of the diffusivities of water vapor and  $CO_2$  (i.e.,  $g_w = ag_c$ ) and  $\frac{c_i}{c_a}$  can be estimated from  $^{13}C$  measurements (Lloyd and Farquhar 1994; Cernusak et al. 2009). Equation (3) shows that WUE is inversely

proportional to  $\frac{c_i}{c_a}$  for a given  $D$ , so that trends in  $\frac{c_i}{c_a}$  as a function of tree size as inferred from  $^{13}\text{C}$  measurements correspond to patterns in water use in terms of WUE. Starting from the WUE formulation in Eq. (3),  $WUE_i$  is obtained as,

$$WUE_i = \frac{A}{g_w} = WUE \times D = \frac{c_a}{a} \left( 1 - \frac{c_i}{c_a} \right). \quad (4)$$

The last step in this derivation is the linkage between  $C$  isotopic discrimination ( $\Delta^{13}\text{C}$ ) and  $\frac{c_i}{c_a}$ . A good approximation for this relation when mesophyll conductance is not limiting is given by (Cernusak et al. 2009 and references therein),

$$\Delta^{13}\text{C} = a + (b - a) \frac{c_i}{c_a}, \quad (5)$$

where  $a = 4.4 \times 10^{-3}$  and  $b = 27 \times 10^{-3}$ . In turn, the discrimination  $\Delta^{13}\text{C}$  is linked to the  $C$  isotope ratio  $\delta^{13}\text{C}$  by the following definition (Barbour et al. 2000; Cernusak et al. 2009),

$$\delta^{13}\text{C} = \frac{\delta^{13}\text{C}_a - \Delta^{13}\text{C}}{1 + \Delta^{13}\text{C}}, \quad (6)$$

where  $\delta^{13}\text{C}_a$  is the isotopic signature of the  $\text{CO}_2$  in the atmosphere. Using Eqs. (5) and (6),  $WUE$  can thus be linked to the measured  $\delta^{13}\text{C}$  via its relation to  $\frac{c_i}{c_a}$  (Eq. 3). Because both  $WUE$  and  $\delta^{13}\text{C}$  decrease with increasing  $\frac{c_i}{c_a}$ , higher  $WUE$  corresponds to less negative (increasing)  $\delta^{13}\text{C}$ .

### Linking water use efficiency to size-dependent traits

To link  $WUE_i$  to plant traits, we start by expressing  $A$  in Eq. (4) as a function of stomatal conductance and photosynthetic capacity. To this aim, the photosynthetic  $C$  demand is defined as a linear function of leaf internal  $\text{CO}_2$  concentration (Hari et al. 1986),

$$A = kc_i, \quad (7)$$

where  $k$  is the carboxylation capacity and the respiration terms have been neglected—a reasonable approximation when focusing on periods of high photosynthetic rates (central part of the day). Combining Eqs. (2) and (7), and eliminating  $c_i$ , a relation between photosynthetic rate and stomatal conductance and photosynthetic capacity is found,

$$A = \frac{kc_a g_w}{ak + g_w}. \quad (8)$$

Substituting Eqs. (8) into (4),  $WUE_i$  is expressed as,

$$WUE_i = \frac{kc_a}{ak + g_w}, \quad (9)$$

where possible effects of plant size could be mediated by  $g_w$  or  $k$ . We thus proceed to link  $g_w$  to hydraulic traits by equating the transpiration rate per unit leaf area in Eq. (1) to the rate of water supply from the stems, also expressed on a per unit leaf area basis (Martinez-Vilalta et al. 2014),

$$E = g_w D = g_{\text{SL}} \frac{\text{SA}}{\text{TLA}} \Delta\psi, \quad (10)$$

where  $g_{\text{SL}}$  is the sapwood area-specific conductance of the whole soil-leaf (SL) continuum (combining soil-to-root and xylem conductances), SA is the sapwood area at the tree base, TLA is the total leaf area, and  $\Delta\psi$  is the water potential difference between the roots and the canopy. This simple approach for modelling tree hydraulics clearly neglects vertical variations in hydraulic traits and the vertical changes in pressure, which might lead to localized cavitation. While this approximation is reasonable for short trees such as willows in SRC systems, it is not suitable as a general description of large trees (Couvreur et al. 2018). Solving Eq. (10) for  $g_{\text{SL}}$ , and inserting the obtained expression in Eq. (9) yields,

$$WUE_i = \frac{kc_a}{ak + g_{\text{SL}} \frac{\text{SA}}{\text{TLA}} \frac{\Delta\psi}{D}}. \quad (11)$$

As a first approximation, some terms in Eq. (11) can be regarded as independent of tree size:  $a = 1.6$  is a constant,  $c_a$  and  $D$  represent environmental conditions, and  $\Delta\psi$  and  $g_{\text{SL}}$  are predicted by metabolic theory to be independent of tree size (West et al. 1999; Savage et al. 2010). The argument in support to this latter claim is based on the assumption that  $\Delta\psi$  is maintained stable during ontogeny, and the theoretical result that both SA and whole canopy transpiration rate scale as  $3/4$  power of plant mass. Because canopy transpiration is given by  $g_{\text{SL}} \text{SA} \Delta\psi$  (Eq. 10), it follows that  $g_{\text{SL}}$  is size-invariant. Therefore, only two terms in Eq. (11) may depend on tree size and can thus affect the  $WUE_i$  as trees grow: the sapwood area to leaf area ratio (SA/TLA) and the carboxylation capacity  $k$  (Fig. 6).

### References

- Albert K, Annighöfer P, Schumacher J, Ammer C (2014) Biomass equations for seven different tree species growing in coppice-with-standards forests in Central Germany. *Scand J For Res*. <https://doi.org/10.1080/02827581.2014.910267>
- Annighöfer P, Mölder I, Zerbe S et al (2012) Biomass functions for the two alien tree species *Prunus serotina* Ehrh. and *Robinia pseudoacacia* L. in floodplain forests of Northern Italy. *Eur J For Res* 131:1619–1635. <https://doi.org/10.1007/s10342-012-0629-2>
- Barbour MM (2007) Stable oxygen isotope composition of plant tissue: a review. *Funct Plant Biol* 34:83. <https://doi.org/10.1071/FP06228>
- Barbour MM, Farquhar GD (2000) Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton



- leaves. *Plant Cell Environ* 23:473–485. <https://doi.org/10.1046/j.1365-3040.2000.00575.x>
- Barbour MM, Fischer RA, Sayre KD, Farquhar GD (2000) Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. *Funct Plant Biol* 27:625–637
- Barbour MM, Walcroft AS, Farquhar GD (2002) Seasonal variation in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of cellulose from growth rings of *Pinus radiata*. *Plant Cell Environ* 25:1483–1499. <https://doi.org/10.1046/j.0016-8025.2002.00931.x>
- Barbour MM, Tcherkez G, Bickford CP et al (2011)  $\delta^{13}\text{C}$  of leaf-respired  $\text{CO}_2$  reflects intrinsic water-use efficiency in barley. *Plant Cell Environ* 34:792–799. <https://doi.org/10.1111/j.1365-3040.2011.02282.x>
- Barigah TS, Saugier B, Mousseau M et al (1994) Photosynthesis, leaf area and productivity of 5 poplar clones during their establishment year. *Ann des Sci For* 51:613–625. <https://doi.org/10.1051/forest:19940607>
- Barnard HR, Brooks JR, Bond BJ (2012) Applying the dual-isotope conceptual model to interpret physiological trends under uncontrolled conditions. *Tree Physiol* 32:1183–1198. <https://doi.org/10.1093/treephys/tps078>
- Beer C, Ciais P, Reichstein M et al (2009) Temporal and among-site variability of inherent water use efficiency at the ecosystem level. *Global Biogeochem Cycles*. <https://doi.org/10.1029/2008GB003233>
- Berlin S, Lagercrantz U, von Arnold S et al (2010) High-density linkage mapping and evolution of paralogs and orthologs in *Salix* and *Populus*. *BMC Genom* 11:129. <https://doi.org/10.1186/1471-2164-11-129>
- Berlin S, Hallingbäck HR, Beyer F et al (2017) Genetics of phenotypic plasticity and biomass traits in hybrid willows across contrasting environments and years. *Ann Bot* 120:87–100. <https://doi.org/10.1093/aob/mcx029>
- Bond-Lamberty B, Wang C, Gower ST (2002) Aboveground and belowground biomass and sapwood area allometric equations for six boreal tree species of northern Manitoba. *Can J For Res* 32:1441–1450. <https://doi.org/10.1139/x02-063>
- Bonos L, Ghelardini L, Weih M (2013) Towards making willows potential bio-resources in the South: Northern *Salix* hybrids can cope with warm and dry climate when irrigated. *Biomass Bioenerg* 51:136–144. <https://doi.org/10.1016/j.biombioe.2013.01.009>
- Brienen RJW, Gloor E, Clerici S et al (2017) Tree height strongly affects estimates of water-use efficiency responses to climate and  $\text{CO}_2$  using isotopes. *Nat Commun* 8:288. <https://doi.org/10.1038/s41467-017-00225-z>
- Broeckx LS, Fichot R, Verlinden MS, Ceulemans R (2014) Seasonal variations in photosynthesis, intrinsic water-use efficiency and stable isotope composition of poplar leaves in a short-rotation plantation. *Tree Physiol* 34:701–715. <https://doi.org/10.1093/treephys/tpu057>
- Buchmann N, Kao W-Y, Ehleringer J (1997) Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah, United States. *Oecologia* 110:109–119. <https://doi.org/10.1007/s004420050139>
- Cabrera-Bosquet L, Albrizio R, Nogue S, Araus JL (2011) Dual  $\Delta^{13}\text{C}/\delta^{18}\text{O}$  response to water and nitrogen availability and its relationship with yield in field-grown durum wheat. *Plant Cell Environ* 34:418–433. <https://doi.org/10.1111/j.1365-3040.2010.02252.x>
- Cernusak LA, Tcherkez G, Keitel C et al (2009) Why are non-photosynthetic tissues generally  $^{13}\text{C}$  enriched compared with leaves in  $\text{C}_3$  plants? Review and synthesis of current hypotheses. *Funct Plant Biol* 36:199. <https://doi.org/10.1071/FP08216>
- Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. *Funct Plant Biol* 30:239. <https://doi.org/10.1071/FP02076>
- Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2002) Improving intrinsic water-use efficiency and crop yield. *Crop Sci* 42:122. <https://doi.org/10.2135/cropsci2002.1220>
- Couvreur V, Ledder G, Manzoni S et al (2018) Water transport through tall trees: A vertically-explicit, analytical model of xylem hydraulic conductance in stems. *Plant Cell Environ*. <https://doi.org/10.1111/pce.13322> (in press)
- Dawson TE, Ehleringer JR (1991) Streamside trees that do not use stream water. *Nature* 350:335–337. <https://doi.org/10.1038/350335a0>
- Dawson TE, Mambelli S, Plamboeck AH et al (2002) Stable isotopes in plant ecology. *Annu Rev Ecol Syst* 33:507–559. <https://doi.org/10.1146/annurev.ecolsys.33.020602.095451>
- Dillen SY, Marron N, Koch B, Ceulemans R (2008) Genetic variation of stomatal traits and carbon isotope discrimination in two hybrid poplar families (*Populus deltoides* “S9-2” x *P. nigra* “Ghoy” and *P. deltoides* “S9-2” x *P. trichocarpa* V24). *Ann Bot* 102:399–407. <https://doi.org/10.1093/aob/mcn107>
- Dongmann G, Nürnberg HW, Förstel H, Wagener K (1974) On the enrichment of  $\text{H}_2^{18}\text{O}$  in the leaves of transpiring plants. *Radiat Environ Biophys* 11:41–52. <https://doi.org/10.1007/BF01323099>
- European Union Monitoring Agricultural Resources (MARS) (1995–2017). <http://agri4cast.jrc.ec.europa.eu/DataPortal/>. Accessed 12 Apr 2017
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol* 33:317–345. <https://doi.org/10.1146/annurev.pp.33.060182.001533>
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 40:503–537. <https://doi.org/10.1146/annurev.pp.40.060189.002443>
- Farquhar GD, Cernusak LA, Barnes B (2007) Heavy water fractionation during transpiration. *Plant Physiol* 143:11–18. <https://doi.org/10.1104/pp.106.093278>
- Fichot R, Chamaillard S, Depardieu C et al (2011) Hydraulic efficiency and coordination with xylem resistance to cavitation, leaf function, and growth performance among eight unrelated *Populus deltoides* x *Populus nigra* hybrids. *J Exp Bot* 62:2093–2106. <https://doi.org/10.1093/jxb/erq415>
- Franks SJ, Weber JJ, Aitken SN (2014) Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evol Appl* 7:123–139. <https://doi.org/10.1111/eva.12112>
- Gago J, Douthe C, Florez-Sarasa I et al (2014) Opportunities for improving leaf water use efficiency under climate change conditions. *Plant Sci* 226:108–119. <https://doi.org/10.1016/j.plantsci.2014.04.007>
- Hari P, Makela A, Korpilahti E, Holmberg M (1986) Optimal control of gas exchange. *Tree Physiol* 2:169–175. <https://doi.org/10.1093/treephys/2.1-2-3.169>
- Hayat A, Hackett-Pain AJ, Pretzsch H et al (2017) Modeling tree growth taking into account carbon source and sink limitations. *Front Plant Sci* 8:1–15. <https://doi.org/10.3389/fpls.2017.00182>
- Huber B (1928) Weitere quantitative Untersuchungen über das Wasserleitungssystem der Pflanzen. *Jahrb Wiss Bot* 67:877–959
- Hunt AG, Manzoni S (2015) The physics of geobiology and geochemistry. In: Networks on networks the physics of geobiology and geochemistry, IOP Concis. Morgan & Claypool, New York, pp 1–14
- Impa SM, Nadarajan S, Boominathan P et al (2005) Carbon isotope discrimination accurately reflects variability in WUE measured at a whole plant level in rice. *Crop Sci* 45:2517–2522. <https://doi.org/10.2135/cropsci2005.0119>



- Johnsen KH, Flanagan LB, Huber DA, Major JE (1999) Genetic variation in growth, carbon isotope discrimination, and foliar N concentration in *Picea mariana*: analyses from a half-diallel mating design using field-grown trees. *Can J For Res* 29:1727–1735. <https://doi.org/10.1139/x99-144>
- Karp A, Shield I (2008) Bioenergy from plants and the sustainable yield challenge. *New Phytol* 179:15–32. <https://doi.org/10.1111/j.1469-8137.2008.02432.x>
- Keitel C, Adams MA, Holst T et al (2003) Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). *Plant Cell Environ* 26:1157–1168. <https://doi.org/10.1046/j.1365-3040.2003.01040.x>
- Kempes CP, West GB, Crowell K, Girvan M (2011) Predicting maximum tree heights and other traits from allometric scaling and resource limitations. *PLoS One*. <https://doi.org/10.1371/journal.pone.0020551>
- Kuzovkina YA, Weih M, Romero MA et al (2008) *Salix*: Botany and Global Horticulture. In: Janick J (ed) Horticultural reviews. Wiley, Hoboken, pp 447–489
- Lamy J, Delzon S, Bouche PS et al (2013) Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. *New Phytol* 201:874–886. <https://doi.org/10.1111/nph.12556>
- Lloyd J, Farquhar GD (1994) C13 discrimination during CO2 assimilation by the terrestrial biosphere. *Oecologia* 99:201–215. <https://doi.org/10.1007/BF00627732>
- Longuetaud F, Piboule A, Wernsdörfer H, Collet C (2013) Crown plasticity reduces inter-tree competition in a mixed broadleaved forest. *Eur J For Res* 132:621–634. <https://doi.org/10.1007/s10342-013-0699-9>
- Martinez-Vilalta J, Poyatos R, Aguadé D et al (2014) A new look at water transport regulation in plants. *New Phytol* 204:105–115. <https://doi.org/10.1111/nph.12912>
- McDowell N, Pockman WT, Allen CD et al (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178:719–739. <https://doi.org/10.1111/j.1469-8137.2008.02436.x>
- McDowell NG, Bond BJ, Dickman LT et al (2011) Relationships between tree height and carbon isotope discrimination. Springer, Dordrecht, pp 255–286
- Medrano H, Flexas J, Galmés J (2009) Variability in water use efficiency at the leaf level among Mediterranean plants with different growth forms. *Plant Soil* 317:17–29. <https://doi.org/10.1007/s11104-008-9785-z>
- Muth CC, Bazzaz F (2003) Tree canopy displacement and neighborhood interactions. *Can J For Res* 33:1323–1330. <https://doi.org/10.1139/x03-045>
- Pellis A, Laureysens I, Ceulemans R (2004) Growth and production of a short rotation coppice culture of poplar I. Clonal differences in leaf characteristics in relation to biomass production. *Biomass Bioenerg* 27:9–19. <https://doi.org/10.1016/j.biombioe.2003.11.001>
- Pucholt P, Sjödin P, Weih M et al (2015) Genome-wide transcriptional and physiological responses to drought stress in leaves and roots of two willow genotypes. *BMC Plant Biol* 15:244. <https://doi.org/10.1186/s12870-015-0630-2>
- Ripullone F, Guerrieri MR, Saurer M et al (2009) Testing a dual isotope model to track carbon and water gas exchanges in a Mediterranean forest. *IForest* 2:59–66. <https://doi.org/10.3832/ifor0491-002>
- Rönnerberg-Wästljung A C, Glynn C, Weih M (2005) QTL analyses of drought tolerance and growth for a *Salix dasyclados* x *Salix viminalis* hybrid in contrasting water regimes. *Theor Appl Genet* 110:537–549. <https://doi.org/10.1007/s00122-004-1866-7>
- Savage VM, Bentley LP, Enquist BJ et al (2010) Hydraulic trade-offs and space filling enable better predictions of vascular structure and function in plants. *Proc Natl Acad Sci* 107:22722–22727. <https://doi.org/10.1073/pnas.1012194108>
- Scheidegger Y, Saurer M, Bahn M, Siegwolf R (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* 125:350–357. <https://doi.org/10.1007/s004420000466>
- Schifman L, Stella JC, Volk TA, Teece M (2012) Carbon isotope variation in shrub willow (*Salix* spp.) ring-wood as an indicator of long-term water status, growth and survival. *Biomass Bioenerg* 36:316–326. <https://doi.org/10.1016/j.biombioe.2011.10.042>
- Schreiber SG, Hacke UG, Hamann A, Thomas BR (2011) Genetic variation of hydraulic and wood anatomical traits in hybrid poplar and trembling aspen. *New Phytol* 190:150–160. <https://doi.org/10.1111/j.1469-8137.2010.03594.x>
- Sellin A (1994) Sapwood–heartwood proportion related to tree diameter, age, and growth rate in *Picea abies*. *Can J For Res* 24:1022–1028. <https://doi.org/10.1139/x94-133>
- Stewart G, Turnbull M, Schmidt S, Erskine P (1995) C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. *Aust J Plant Physiol* 22:51. <https://doi.org/10.1071/PP950051>
- Strauss-Debenedetti S, Bazzaz FA (1991) Plasticity and acclimation to light in tropical Moraceae of different successional positions. *Oecologia* 87:377–387. <https://doi.org/10.1007/BF00634595>
- Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *J Exp Bot* 49:419–432. [https://doi.org/10.1093/jxb/49.Special\\_Issue.419](https://doi.org/10.1093/jxb/49.Special_Issue.419)
- Toillon J, Rollin B, Dallé E et al (2013) Variability and plasticity of productivity, water-use efficiency, and nitrogen exportation rate in *Salix* short rotation coppice. *Biomass Bioenerg* 56:392–404. <https://doi.org/10.1016/j.biombioe.2013.05.017>
- Vertessy R, Benyon RG, O’Sullivan SK, Gribben PR (1995) Relationships between stem diameter, sapwood area, leaf area and transpiration in a young mountain ash forest. *Tree Physiol* 15:559–567. <https://doi.org/10.1093/treephys/15.9.559>
- Waring RH, Schroeder PE, Oren R (1982) Application of the pipe model theory to predict canopy leaf area. *Can J For Res* 12:556–560. <https://doi.org/10.1139/x82-086>
- Weih M, Nordh N-E (2005) Determinants of biomass production in hybrid willows and prediction of field performance from pot studies. *Tree Physiol* 25:1197–1206. <https://doi.org/10.1093/treephys/25.9.1197>
- Weih M, Rönnerberg-Wästljung A-C, Glynn C (2006) Genetic basis of phenotypic correlations among growth traits in hybrid willow (*Salix dasyclados* x *S. viminalis*) grown under two water regimes. *New Phytol* 170:467–477. <https://doi.org/10.1111/j.1469-8137.2006.01685.x>
- West GB, Brown JH, Enquist BJ (1999) A general model for the structure and allometry of plant vascular systems. *Nature* 400:664–667. <https://doi.org/10.1038/23251>
- Yang Yu B, Elbuken C, Ren CL, Huissoon JP (2011) Image processing and classification algorithm for yeast cell morphology in a microfluidic chip. *J Biomed Opt* 16:066008. <https://doi.org/10.1117/1.3589100>
- Yin C, Duan B, Wang X, Li C (2004) Morphological and physiological responses of two contrasting Poplar species to drought stress and exogenous abscisic acid application. *Plant Sci* 167:1091–1097. <https://doi.org/10.1016/j.plantsci.2004.06.005>
- Zhang X, Zang R, Li C (2004) Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. *Plant Sci* 166:791–797. <https://doi.org/10.1016/j.plantsci.2003.11.016>