



# Evaluating growth and intrinsic water-use efficiency in hardwood and conifer mixed plantations

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## Abstract

**Key message** *Juglans*, *Fraxinus*, *Quercus* and *Pinus* species seem to better maximize the carbon–water ratio providing useful indications on species selection for forestry plantations in areas with increasing drought risk.

**Abstract** Maximizing carbon sequestration for a given water budget is extremely important in the contest of climate change in the Mediterranean region, which is characterized by increasing temperatures and rising water stress. This issue is fundamental for plantation stands, where limited water availability during the growing season reduces CO<sub>2</sub> assimilation and, consequently, tree growth. In this study, the main objective was to investigate the performances in terms of carbon–water balance of conifer (*Pinus halepensis* and *Cupressus sempervirens*) and hardwood (*Quercus robur*, *Juglans regia*, *Fraxinus excelsior* and *Populus* spp.) mixed plantations. To this aim, we used carbon isotope signatures to evaluate the intrinsic water-use efficiency (*iWUE*) and the species-specific relationship between basal area increments (*BAI*) and *iWUE*. At the species level, the highest *iWUE* values corresponded to the lowest carbon accumulation in terms of *BAI*, for water-saving species such as *Cupressus*. Conversely, *Populus* had the lowest *iWUE* and the highest *BAI* accumulation. *Juglans*, *Fraxinus*, and *Pinus* showed the most balanced ratio between *BAI* and *iWUE*. Overall, no clear correlation of *iWUE* and *BAI* was evident within all species, except for *Populus* and *Cupressus*. Considering projected aridification and increased temperatures that will negatively impact the growth, our data suggest that *Pinus*, for conifers, and *Quercus*, *Juglans*, *Fraxinus* for hardwood species should be preferred when choosing species for forestry plantation, as they performed better in terms of *BAI* and *iWUE* ratio.

**Keywords** Carbon isotope composition · Forest productivity · Plantations · Tree growth · Water-use efficiency

## Introduction

Forests contribute to the reduction of atmospheric CO<sub>2</sub> concentrations and help to mitigate climate change (Grassi et al. 2017). In recent decades, increasing temperatures,

water scarcity and the highest frequency of extreme weather events have been observed especially in Mediterranean areas (Giorgi and Lionello 2008; Ripullone et al. 2009a). In this scenario, forest-based mitigation strategies—including afforestation, reforestation and reducing deforestation—are important to reduce atmospheric CO<sub>2</sub> and increase C sequestration (Reyer et al. 2009). These practices favor rapid tree growth and carbon sequestration in post-harvest wood products (Harmon and Marks 2002; Kobziar and Stephens 2006; Krankina and Harmon 2006).

Some forestry practices that apparently mitigate global warming such as afforestation activities may actually intensify water use and, therefore, deplete available water reserves (Unkovich et al. 2003; Dias de Oliveira et al. 2005). For example, plantations composed of highly productive species used for bioenergy production generally use much more water than the species naturally occurring in these areas (Jackson et al. 2005). Moreover, certain management

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practices, such as the use of nitrogenous fertilizers for agriculture and forestry, in some cases may also reduce water-use (Lauteri et al. 1997; Farley et al. 2005; Battipaglia et al. 2017), likely due to interacting effects with drought or nutrient imbalance, leading to interspecific differences in the water-use responses to nitrogen fertilization (Smith and van den Driessche 1992; Ripullone et al. 2004).

Biomass production and intrinsic water-use efficiency (*iWUE*, as the ratio between carbon assimilation and water transpired) are important parameters that should be considered when choosing tree species for plantations. Although rapid biomass accumulation in fast-growing trees (short rotation forestry) is important in the interests of resource management and environmental sustainability, water consumption (related to the tree water-use and water lost by transpiration) should also be taken into account. Therefore, forest management should preferably focus on species and silvicultural practices that increase C sequestration without reducing groundwater reserves (Unkovich et al. 2003; Dias de Oliveira et al. 2005). Consequently, the choice of trees for use in plantations must factor in both the growth potential and the water use of each candidate species.

Sustainable water management in forested areas (i.e. afforestation, plantation, short rotations) is possible when the carbon to water balance ratio is considered. Under limited water conditions which frequently occur in the Mediterranean, plants partially close their stomata to save water and maintain their leaf water potential within a safety range to prevent cavitation (Ripullone et al. 2009a). Although stomatal control favours water saving, it has a negative effect on plant carbon uptake by down-regulating the CO<sub>2</sub> assimilation (Jarvis and Davies 1998). The intrinsic water-use efficiency (*iWUE*), assessing the ratio between CO<sub>2</sub> assimilation and stomatal conductance, is a key parameter in drought-prone areas (Ripullone et al. 2009b; Altieri et al. 2015). Understanding species-specific differences in *iWUE* may help to elucidate how plantation and afforestation stands respond to drought occurrence.

The carbon isotope composition ( $\delta^{13}\text{C}$ ) is a useful proxy to improve understanding of forest responses to climate-changing conditions over time (McCarroll and Loader 2004); it contributes to determining whether a plant species saves water as indirectly related to water-use efficiency (Farquhar et al. 1989a). There is a relationship between *iWUE* and  $\delta^{13}\text{C}$  because of their independent linkages to the ratio of internal to ambient CO<sub>2</sub> concentrations ( $c_i/c_a$ ). Studies have reported an increase in *iWUE* around the globe (Loader et al. 2011; Marchand et al. 2020) and Mediterranean areas (Andreu-Hayles et al. 2011), even if higher *iWUE* does not always translate into enhanced tree growth (Peñuelas et al. 2011; Silva and Anand 2013; and others). In open field studies, no clear pattern emerged, reporting both active and passive plant response: in the first case  $c_i$  increases more

slowly than  $c_a$  due to photosynthetic plasticity, resulting in higher *iWUE* (Duquesnay et al. 1998; Feng 1999; Peñuelas et al. 2008; Guerrieri et al. 2019), while the second case no changes in the  $c_a-c_i$  are highlighted in response to increased atmospheric CO<sub>2</sub> concentration ( $c_a$ ), which resulted in no improvement of the *iWUE* (Marshall and Monserud 1996). Saurer et al. (2014) suggested that site-specific conditions are relevant due to enhanced *iWUE* in temperate forests of central Europe following a decreasing soil–water availability as a consequence of the ongoing climate change. However, multi-species studies carried out in Mediterranean areas focusing on variations in terms of *iWUE* growth and plant ecophysiological adjustments are lacking.

We hypothesized that the ongoing climate change should favour *iWUE* but disadvantage the growth as a consequence of enhancing drought events which negatively affect photosynthetic activity in both conifer and hardwood studied species. To test this idea, we investigated the magnitude of the co-variations in *BAI* and *iWUE* across species growing in forestry plantations. We considered contrasting environmental conditions in an afforested stand with conifer species (*Pinus halepensis* and *Cupressus sempervirens*) and in a coexisting stand with hardwood species (*Quercus robur*, *Juglans regia*, *Fraxinus excelsior*, and *Populus* spp), among the most common species used in the past for plantations in Italy. Specifically, we evaluated: (1) the growth in terms of basal area increment (*BAI*); (2) the intrinsic water-use efficiency (*iWUE*) as assessed by  $\delta^{13}\text{C}$  in annual tree rings, and (3) the short-term species-specific ecophysiological adjustments, derived from *iWUE*.

## Materials and methods

### Study sites

The experiments were conducted at Camisano in northern Italy (*Site 1*) and at Gallipoli Cognato and Piccole Dolomiti Lucane Regional Parks in southern Italy (*Site 2*), Table 1.

*Site 1* lies in the Padan basin and belongs to a flat area located ~30 km east of Milan in northwestern Italy. The climate is classified as mild continental with an average total annual rainfall of 1050 mm, almost equally distributed throughout the year. Maximum precipitation occurs during fall and winter and the average temperature is 10 °C. The natural vegetation of the Padan basin is a mixed hardwood forest consisting of *Quercus robur*, *Populus* spp., *Carpinus* spp., *Alnus* spp., *Salix* spp., *Fraxinus* spp., and other trees indigenous to central Europe. The site is a mixed-species plantation established at the beginning of 1997 and covering 2.8 ha. The above-mentioned species, ecologically and economically important (Colpi et al. 1999), are widely employed in plantations in Italy especially from the 1960s

**Table 1** Main characteristics of sites and sampled trees

	Lat	Long	Altitude (m asl)	Sampled spp.	dbh	se
Site 1	45° 27' 08.7" N	9° 45' 40.7" E	92	<i>Juglans</i>	34.83	1.47
				<i>Fraxinus</i>	25.75	0.83
				<i>Quercus</i>	23.58	0.35
				<i>Populus</i>	32.00	0.95
Site 2	40° 32' 44.46" N	16° 5' 17.66" E	550	<i>Pinus</i>	21.32	1.83
				<i>Cupressus</i>	20.42	1.91

to 1990s of the last century covering more than one million hectares.

The plantation consisted of a main plot (*P1*) (~2.8 ha) with coexisting coetaneous species (*Quercus robur*, *Fraxinus excelsior*, and *Juglans nigra*), and monospecific *Populus alba* (clone *a4*) rows (*P2*), surrounding the main plot. The trees in *P1* and *P2* were planted in straight rows spaced 5 m apart corresponding to a density of ~400 trees per hectare. The crop cycle in *P1* and *P2* is different: hardwood species in *P1* and *Populus* trees in *P2* are managed as arboriculture and short rotation (for biomass energy), respectively. This implies a shorter cultivation cycle for *Populus*, that showed a different age (9 years old) compared to the other hardwood species (16 years old) sampled in *P1*. Nevertheless, *Populus* trees were considered as widely employed species representing a useful reference for biomass production and water use.

*Site 2* belongs to a mountain stand located in the South of Italy; the bedrock is an arenaceous stone “*Flysch di Gorgoglione*” (alternating sandstone, marl, and clay) and the soil texture is mainly sand. The climate is the Mediterranean with a mean total annual rainfall of 687 mm, concentrated in fall and winter, and an average temperature of 12 °C. There is a dry period from June to September during which precipitation is < 100 mm. The stand studied here (~150 ha) was planted at the end of the 1960s with trees in rows 3 m apart with an overall stand density of ~1200 trees ha<sup>-1</sup>. The stand is dominated by *Pinus halepensis* (P) and *Cupressus glabra* (C); together, they comprise ~90% of the vegetation cover and their presence is shared at approximately 50%. In addition, other species such as *Phillyrea angustifolia*, *Pistacia lentiscus*, and *Ostrya carpinifolia* are present. The selected stands belong to a managed area where during 2000s ordinary thinning, with a reduction of 20% of standing biomass, were applied.

## Sampling

In spring 2015, 7 and 15 healthy dominant trees representative of each considered species were sampled at the plantation (*Site 1*) and afforestation (*Site 2*) stands, respectively. Two wood cores were extracted with an incremental borer at collar level and breast height for each tree at *Site 1* and *Site 2*, respectively.

Samples were air-dried and polished with a scalpel until the lumens of the vessels and tracheids were clearly visible. Tree-ring series were visually cross-dated and ring widths were measured at a 0.01-mm resolution with an incremental measuring table Lega SMIL3 (Corona et al. 1989). Chronologies were checked within a species and site with Cofecha (Holmes 1983).

In our study, we considered even-aged plantations with poplar trees, managed as short rotation forestry that were slightly younger compared to the hardwood tree species sampled at *Site 1*. Therefore, to evaluate tree productivity in the selected stands by accounting for the age-effect, the raw chronologies were converted into tree basal area increments (BAI) as follows:

$$BAI_t = \pi r_t^2 - \pi r_{t-1}^2$$

where BAI at year *t* is the annual ring area; and *r<sub>t</sub>* and *r<sub>t-1</sub>* are the stem radii at the end and beginning of the annual increment, respectively. BAI eliminates the age effect associated with stem geometry, reduces low-frequency variability, and obviates the need for detrending (Biondi 1999).

## Stable C isotope analysis

For each species, five trees were selected for isotopic analyses. From each dated tree core, single rings were separated with a blade cutter. Each sample was ground in a centrifugal mill (ZM 1000, Retsch, Germany) with a 0.5-mm mesh size to ensure homogeneity. Isotopic analysis was performed on whole wood samples in hardwood species since recent studies have shown that the use of whole-wood isotope values is justified for ecophysiological and dendrochronological studies that analyze the response of trees to environmental changes recorded within the sapwood in a relatively short-term period (Borella et al. 1998; Harlow et al. 2005; Riechelmann et al. 2016). However, in conifer species isotopic analysis was performed on cellulose samples, to avoid biases on isotope value due to contamination by other wood extractives (Borella et al. 1998; D'Alessandro et al. 2004). For the extraction of cellulose a two-step digestion process was followed (Boettger et al. 2007; Battipaglia et al. 2008). The method is based on a double step digestion: first step

was performed for the extraction of resin, fatty acids, etheral oils and hemicellulose with a solution of 5% NaOH for 2 h at 60 °C—this operation was repeated twice. In the second step, lignin was extracted with a 7% NaClO<sub>2</sub> solution for a minimum of 36 h at 60 °C. Because the solution is only reactive for about 10 h, it was changed daily and refilled as necessary. This step was repeated until the sample was “white”, which is a characteristic that can be determined by experience or by comparison with commercial cellulose (Boettger et al. 2007). Finally, samples were washed three to four times with boiling distilled water (until pH = 7 ± 1) and dried overnight at 50 °C.

0.06 mg wood and alpha-cellulose material were weighed in tin capsules, for hardwood and conifer species, respectively. Stable C isotope composition was measured at the IRMS-SUN Laboratory (Caserta, Italy) by a combustion in an elemental analyzer (Carlo Erba, 1110 Milano, Italy) connected via a CONFLO II interface (Thermo Finnigan, Breen, Germany) to an isotope ratio mass-spectrometer (Delta V Advantage, Thermo Electron Corporation, Bremen Germany) operating in the continuous flow mode. Isotopic compositions are expressed in delta notation (‰) relative to an accepted reference standard: Vienna PeeDee belemnite for carbon isotope values. The standard deviation for the repeated analysis of an internal standard (commercial cellulose) was < 0.01‰.

### Calculation of *iWUE* from C isotope ratios

Carbon isotope composition in tree rings ( $\delta^{13}C$ ) reflects the variations in the CO<sub>2</sub> concentration ratio between the leaf intercellular spaces and the atmosphere ( $c_i/c_a$ ). These are related to changes in carbon assimilation ( $A$ ) or stomatal conductance ( $g_s$ ). A simplified version of the Farquhar equation (Farquhar et al. 1989b) determines the  $\delta^{13}C$  in plant material ( $\delta^{13}C_p$ ) as follows:

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a) \frac{c_i}{c_a} \quad (1)$$

where  $\delta^{13}C_a$  and  $c_a$  indicate the isotopic signature of atmospheric CO<sub>2</sub>,  $a$  is the fractionation against <sup>13</sup>CO<sub>2</sub> during diffusion through stomata (4.4%), and  $b$  is the fractionation during carboxylation (27%) by the CO<sub>2</sub>-fixing enzyme rubisco. In the *iWUE* calculation, the mesophyll conductance of CO<sub>2</sub> (Seibt et al. 2008) and post-photosynthetic processes that may potentially affect tree-ring  $\delta^{13}C$  (Gessler et al. 2014; Frank et al. 2015) were not considered. Since  $c_i/c_a$  and  $\delta^{13}C$  are reciprocally linked, the latter is indicative of changes in the *iWUE*, which is the ratio between  $A$  and the stomatal conductance to water vapor ( $g_{H_2O}$ ) according to Ehleringer et al. (1993):

$$WUE_i = \frac{A}{g_{H_2O}} = \frac{(c_a - c_i)}{1.6} \quad (2)$$

From Eq. (1),  $c_i$  is calculated as follows:

$$c_i = c_a \frac{\delta^{13}C_a - \delta^{13}C_p - a}{b - a} \quad (3)$$

Therefore the Eq. (2) can be solved as follows:

$$WUE_i = \frac{A}{g_s} = \frac{c_a - c_i}{1.6} = \frac{c_a}{1.6} \times \left( \frac{b - \delta^{13}C_a + \delta^{13}C_p}{b - a} \right) \quad (4)$$

where  $\delta^{13}C_a$  and  $c_a$  values were obtained from Mauna Loa records (Keeling et al 2001); 1.6 is the molar diffusivity ratio of CO<sub>2</sub>–H<sub>2</sub>O (i.e.,  $g_{CO_2} = g_{H_2O}/1.6$ ); the values  $a$  and  $b$  are known,  $c_i$  is calculated from Eq. (3),  $\delta^{13}C_p$  is the carbon isotope composition measured in tree rings.

### Statistical analyses

The six tree species sampled were subdivided into the hardwood species *Juglans nigra*, *Quercus robur*, *Populus* spp., and *Fraxinus excelsior* (Site 1) and the conifers *Pinus halepensis* and *Cupressus glabra* (Site 2). *BAI* data were log-transformed before analysis to meet the normality assumptions. The *LMM* was fitted to assess the effect of age, species, *iWUE* and their interactions (fixed factors) on *BAI*, where individual trees were considered as random effects to account for repeated measures within a site. Following Zuur et al. (2009), the most parsimonious models were selected starting with a saturated model where the fixed component contained all explanatory variables and their possible interactions, using the ‘nlme’ package (Pinheiro et al. 2020) from R environment v.4.0.0 (R Core Team 2020). Fixed terms were centered and scaled to improve parameter estimates and allow direct comparisons of the regression coefficients. We then optimized the random-effect structure of the model, testing whether including extra random-effect terms (i.e., random slopes) for tree ID improved the fit of the model; different random structures were compared through a Likelihood Ratio Test (LRT, which approximately follows a chi-square distribution; Zuur et al. 2009). When comparing saturated models that varied in their random structure but not fixed effects, the models were fit using restricted maximum likelihood (REML) to avoid biased estimators for the variance terms. The fixed-effect structure was optimized by fitting the model with Maximum Likelihood (ML), rather than REML, to prevent biased fixed-effect parameter estimates. We conducted AICc values (Akaike Information Criterion corrected for sample size) based on multi-model inference using the “MuMIn” dredge function (Barton and Barton

2020) to run a complete set of models with all possible combinations of the fixed effects, with  $\Delta\text{AICc} < 2$  interpreted as substantial support that the model belongs to the set of ten best models,  $\Delta\text{AICc}$  of 4–7 corresponding to less support and  $\Delta\text{AICc} > 10$  treated as providing no support that the model belongs to the best (Burnham and Anderson 2002). Finally, models were refitted with REML to estimate model parameters. Marginal and conditional  $R^2$  scores (Nakagawa and Schielzeth 2013) were calculated to examine the variation explained by models using the “*r.squaredGLMM*” function in the “*MuMIn*” package. The residual diagnosis was performed to check the validity of the model assumptions (normality and homoscedasticity of residuals). Subsequently, the *lstrends* function from the ‘*lsmmeans*’ R package (Lenth 2016) was used to estimate and compare, via Tukey post-hoc test, species-specific slopes of fitted lines.

For each species, we further evaluated the temporal pattern of *iWUE* components (i.e.  $c_i$ ,  $c_i/c_a$ ,  $c_a-c_i$ ), by inspecting the slope of the regression line via LMMs where cambial age was included as fixed-term and sampled trees as random. The models were then parametrically bootstrapped 1000 times using the *bootMer* function in package ‘*lme4*’ (Bates et al 2015) from which the predicted median and 95% confidence interval of the beta estimates were calculated.

## Results

### Basal area increment and intrinsic water-use efficiency

In general, a substantial range of variation of *iWUE* and *BAI* was observed (Fig. 1). For all hardwood species, except *Populus*, different *BAI* was measured for similar *iWUE* distribution ranges. Conifers showed contrasting differences between the recorded variables, i.e., *Cupressus* showed greater water-use efficiency, but was less productive in terms of tree growth than *Pinus*.

The hardwood species all had similar annual *iWUE* during 1996–2010, except for *Populus* (Fig. 1a). The *iWUE* for the conifers showed higher interannual variability compared to the considered hardwood species (coefficient of variation was 12.5% and 10.4% for conifers and hardwood, respectively). *BAI* and *iWUE* variations at *Site 2* were observed in correspondence to the thinning operations in both *Cupressus* and *Pinus* stands, which likely occurred in 2003 and 2009 (Fig. 1). The *iWUE* for *Pinus* was significantly lower than *Cupressus* (Fig. 1b); overall, *Cupressus* exhibited the lowest *BAI* (Fig. 1c) whereas *Populus* the highest ( $\sim 2500 \text{ mm}^2$ ), which was also the youngest sampled tree species at *Site 1* (Fig. 1c).

### The role of *iWUE* in determining *BAI*

Species characterized by different strategies for achieving high/low *BAI* in relation to high/low values of *iWUE* were depicted (Fig. 2a). In particular, among hardwood species, *Populus* showed the highest *iWUE* value while *Fraxinus* displayed the lowest value. Between conifer species, *Cupressus* showed the lowest and *Pinus* the highest *iWUE* value per *BAI*. Figure 2b describes the slopes of the species of *BAI* on *iWUE*, whereas the model structures, covariates with model coefficients, standard errors, and significance levels are summarized in the Supplementary Table S1. In particular, the selected model showed that 71% of the total variance was attributable to the fixed effects whereas less than 1% of the variance was attributable to variation between trees. While no evidence for a main effect of *iWUE* on *BAI* was found, differences among species (Supplementary Table S2) are evident where the slopes of the relations with *BAI* of *Populus* and *Pinus* are significantly higher and lower respectively compared to the main effect (i.e. *Cupressus*), while an opposite slope was observed for *Quercus* (Fig. 2b). As expected, tree age had a negative influence on *BAI*.

### Variations in the *iWUE* components

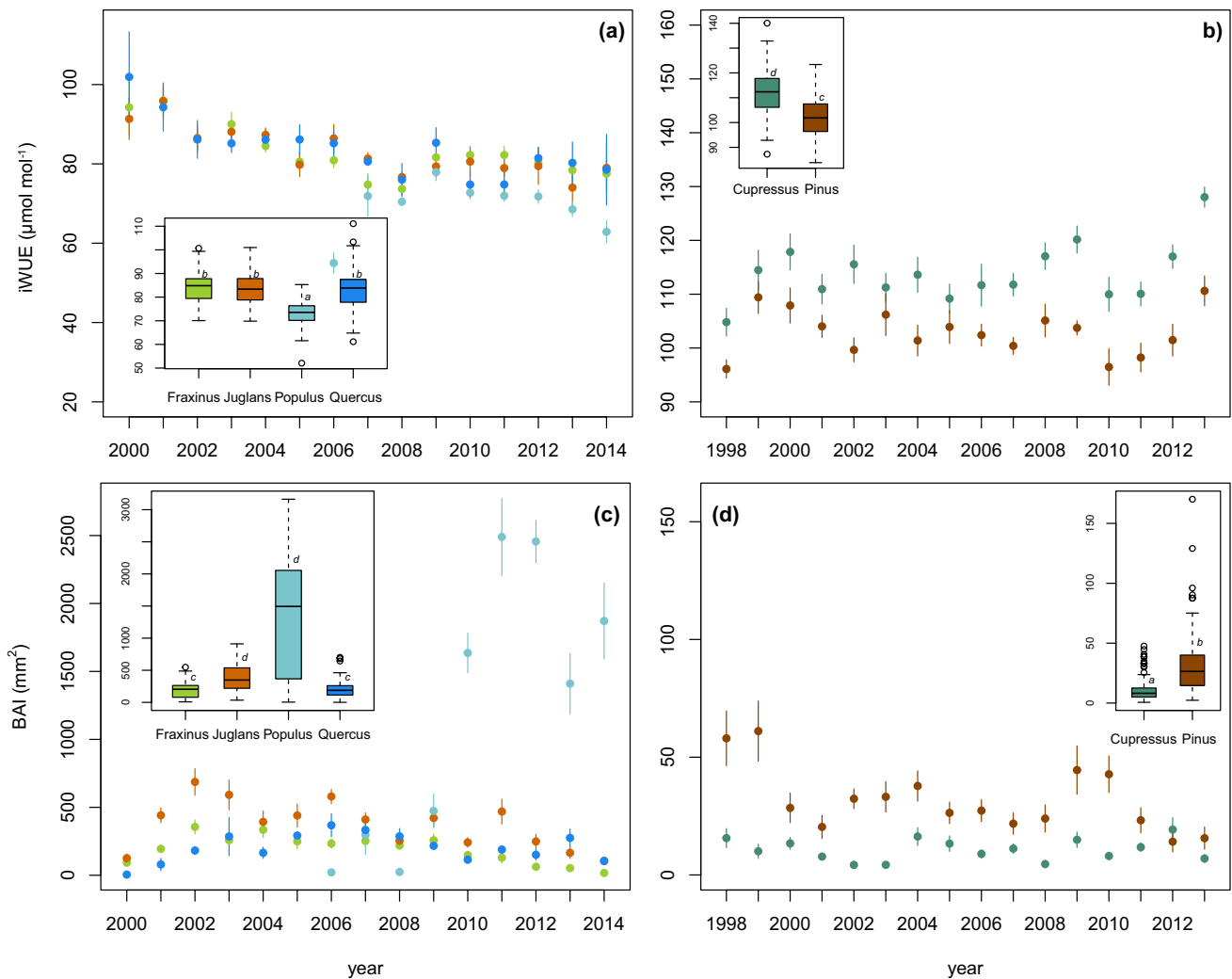
In general increasing  $c_i$  and  $c_i/c_a$  trends were observed with differential *iWUE* trends, as assessed by  $c_a-c_i$  temporal variations (Table 2). In particular, the following main results for  $c_a-c_i$  were observed: a significant increasing trend for *Cupressus* and a significant decreasing trend for *Fraxinus*, *Juglans*, and *Quercus* species; relatively decreasing, and non-significant trends for *Pinus* and *Populus* species. Non-significant variations for  $c_i/c_a$  were recorded for *Cupressus* and *Pinus*, respectively, whereas positive significant trends were recorded for *Fraxinus*, *Juglans*, *Populus*, and *Quercus* species.

Overall  $c_i/c_a$  ratios varied among species showing lower values in conifers than hardwood species (Fig. 3a); a consistent  $c_i$  adjustment with respect to the increasing  $c_a$  was evident in all considered species, although a short-term period was considered (Fig. 3b).

## Discussion

In this study, we analyzed the *iWUE* and *BAI* in a range of tree species, mostly used in Italy in former forestry plantation practices to provide functional-based indications on tree species selection.

The observed strategies for achieving (relatively) high/low *iWUE* (Fig. 2a) are closely related to differences in physiological mechanisms among species. Indeed, different *iWUE* levels may be linked to a greater overall  $\text{CO}_2$



**Fig. 1** Temporal variation in intrinsic water use efficiency (*iWUE*, upper panels) and basal area increment (*BAI*, lower panels) for plantation (**a, c**) and afforestation stands (**b, d**) over the studied period. Points with different colors represent means of different species in each year; bars are standard errors. Insets represent boxplots of species-specific *iWUE* and *BAI*. Each box represents the 75th–25th per-

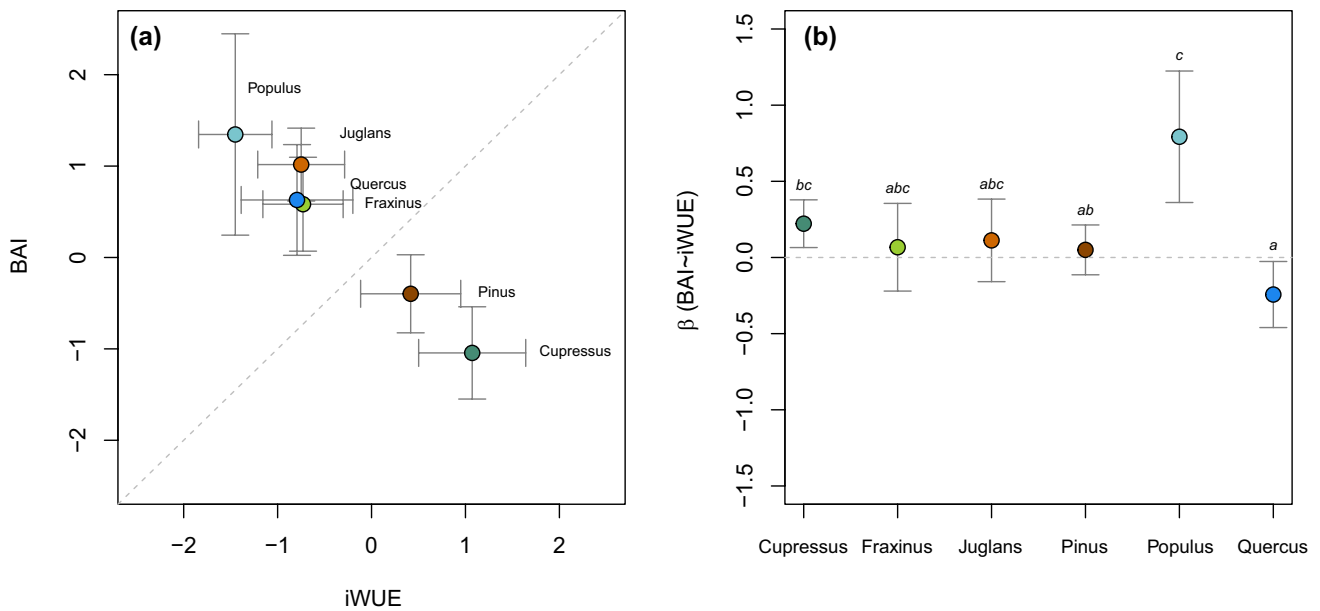
centiles, the bold line shows the median, upper and lower marks are the largest to smallest observation values which are less than or equal to the upper and lower quartiles plus 1.5 times the length of the interquartile range. Circles outside the lower–upper mark range represent outliers. Different letters indicate significant differences among species ( $P < 0.05$ ) Tukey’s pairwise post-hoc comparisons

assimilation capacity of some species or a better stomatal control in other species. *Populus* was distinct from the other species due to its *iWUE* comparatively low coupled to a high growth rate, whilst *Juglans* and *Fraxinus* appeared to have reached a better carbon–water balance, although showing a weak association between considered variables (Fig. 2b). On the contrary, the opposite associations between *BAI* and *iWUE* reported for *Quercus* may be explained by a greater stomatal control compared to the other coexisting species i.e. avoiding water loss by stomatal closure and, consequently, enhancing *iWUE* to the detriment of growth. Similar findings have also been reported in previous studies in both seedlings, under controlled environmental conditions (Picon et al. 1996), and adult trees, growing in natural forests

subjected to dieback phenomena not far from experimental *Site 1* (Colangelo et al. 2018).

One of our objectives was to understand the magnitude of the co-variations in *BAI* and *iWUE* across species growing in a forestry plantation. When considering growth (in terms of *BAI*) as explained by plant ecophysiological trait (*iWUE*), age and species, our results did not support evidence for a general trade-off between annual growth and *iWUE*. A flat or weak relationship was observed for *Juglans*, *Fraxinus*, and *Pinus* species; positive relationships for *Populus* and *Cupressus* while a negative relationship was found for *Quercus* (Fig. 2b).

This general uncoupling between *iWUE* and *BAI* has been widely observed in literature. Indeed, several studies



**Fig. 2** The relationship between *BAI* and *iwUE* is represented in both panels. In **a** circles and bars represent mean values and standard deviations for each investigated tree species; the dashed lines shows 1:1 relationship. **b** Shows comparison via Tukey post-hoc test

of species-specific slopes of the relationship *BAI*–*iwUE* from fitted LMM. Different letters indicate significant differences among species at  $P \leq 0.05$

**Table 2** Observed temporal patterns in components of intrinsic water-use efficiency

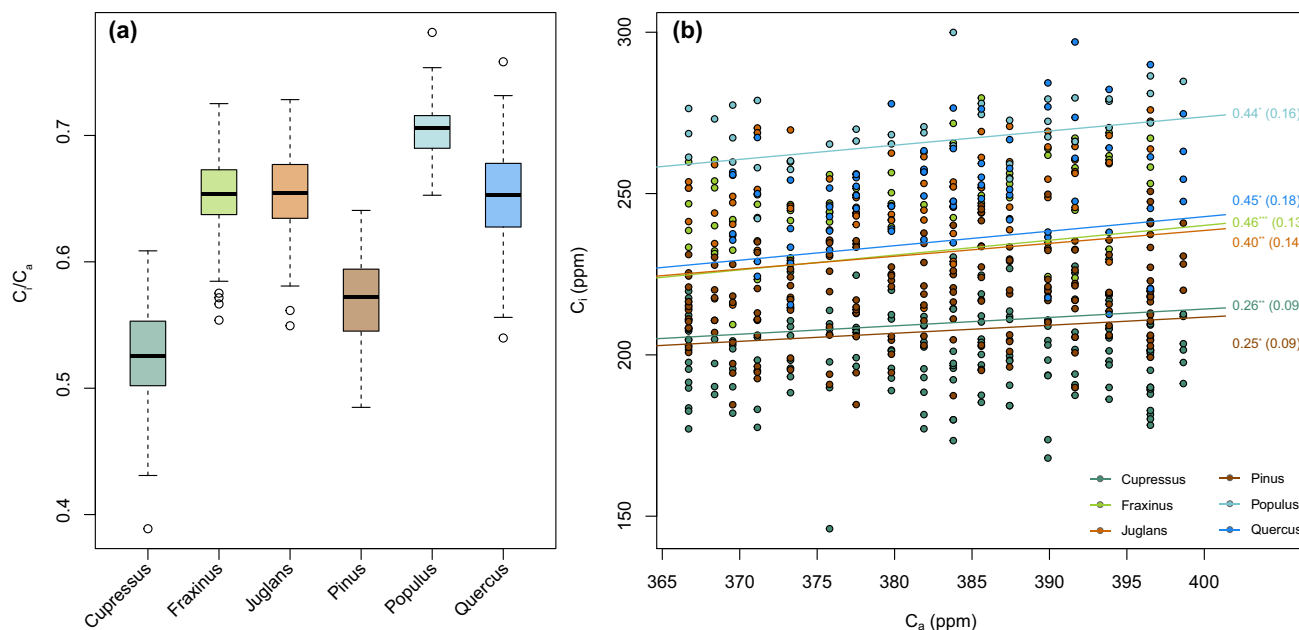
Species	Parameters					
	$c_i$		$c_i/c_a$		$c_a - c_i$	
	$\beta$	CI	$\beta$	CI	$\beta$	CI
<i>Cupressus</i>	–0.001	(–0.014 to 0.011)	–0.010	(–0.023 to 0.001)	<b>0.019</b>	<b>(0.005–0.032)</b>
<i>Fraxinus</i>	<b>0.063</b>	<b>(0.0418–0.085)</b>	<b>0.062</b>	<b>(0.040–0.082)</b>	–0.060	(–0.081 to 0.038)
<i>Juglans</i>	<b>0.0733</b>	<b>(0.0524–0.095)</b>	<b>0.070</b>	<b>(0.048–0.092)</b>	–0.065	(–0.089 to –0.042)
<i>Pinus</i>	<b>0.016</b>	<b>(0.0061–0.026)</b>	0.009	(–0.001 to 0.020)	–0.005	(–0.016 to 0.006)
<i>Populus</i>	<b>0.043</b>	<b>(0.004–0.083)</b>	<b>0.015</b>	<b>(–0.033 to 0.064)</b>	–0.019	(–0.073 to 0.029)
<i>Quercus</i>	<b>0.0789</b>	<b>(0.050–0.106)</b>	<b>0.064</b>	<b>(0.037–0.091)</b>	–0.055	(–0.082 to 0.027)

$\beta$  values correspond to the bootstrapped slope of the fitted LMMs with the cambial age as covariate and tree ID set as random. Bold values indicate the statistical significance at  $P \leq 0.05$ ; the 95% CI, estimated confidence intervals in parenthesis

report that the enhanced *iwUE* observed in the last century (Andreu-Hayles et al. 2011; Leonardi et al. 2012; Frank et al. 2015; Marchand et al. 2020) did not translate into an increase in tree growth (Peñuelas et al. 2011; Silva and Anand 2013; Lévesque et al. 2014). Moreover, site-specific studies are often carried out in open-fields along a latitudinal gradient to appreciate species-specific adaptations to changing climatic conditions by evaluating plant physiological traits adjustments (Guerrieri et al. 2019; Fu et al. 2020). In general, contrasting relationships between *BAI* and *iwUE*, e.g. a negative relationship for conifer whereas positive relationships for hardwood species (*Scots pine* and *European beech* in González de Andrès et al. 2018) were evident; no

common patterns were observed when intra-specific comparisons were carried out (González-Muñoz et al. 2015) and very few studies have been carried out on plantations, i.e. clones of *Populus* (Rasheed et al. 2019). A recent review (Walker et al. 2020) suggested a major increase in *iwUE* in water-limited regions where long-term transpiration is primarily precipitation driven (i.e. plants use the available water).

Most species considered in our study did not report substantial time-related enhanced *BAI* and *iwUE*, showing rather flat or even negative trends (Table 2). This is in contrast with the latest literature (Peñuelas et al 2008; Battipaglia et al. 2013; Guerrieri et al. 2019; Walker et al.



**Fig. 3** **a** Boxplots of ratio between intercellular to ambient carbon dioxide concentration for considered species. Each box represents the 75th–25th percentiles, the bold line shows the median, upper and lower marks are the largest to smallest observation values which are less than or equal to the upper and lower quartiles plus 1.5 times the length of the interquartile range. Circles outside the lower–upper mark range represent outliers. **b** Temporal species-specific relation-

ships between ambient ( $c_a$ ,  $x$  axis) and intercellular ( $c_i$ ,  $y$  axis) carbon dioxide concentration; lines draw each predicted LMMs as a function of  $c_a$  and cambial age as covariates and tree ID set as random; slope coefficients and their standard error of coefficients (in bracket) are reported nearby fitted lines. \* and \*\* indicate statistical significance for  $P < 0.05$  and  $P < 0.01$ , respectively

2020) where an increase in  $iWUE$  is reported. No evidence of increased carbon uptake per unit water loss has been recognized, in agreement with literature studies (Huang et al. 2007; Peñuelas et al. 2008, 2011; Andreu-Hayles et al. 2011; Franks et al. 2013). This could reflect a water-saving strategy in which the stomata are closed, thereby enhancing  $iWUE$  (Battipaglia et al. 2013) or even a climate-related effect, indeed, in some cases, the trade-off carbon uptake per water loss is strongly dependent on the occurrence across the years of favorable/unfavorable climatic conditions (Granda et al. 2014).

### Short-term species-specific adjustments

Even short-term variations in the ratio and difference of intercellular to ambient  $\text{CO}_2$  concentrations can indicate species-specific acclimations to current environmental conditions. According to Saurer et al. (2004), we could observe three scenarios with respect to the possible responses of  $c_i$  to increasing  $c_a$ : (a)  $c_i/c_a$  remains constant when the tree actively controls  $c_i$ ; (b) the increments in  $c_i$  and  $c_a$  are the same and  $c_i$  passively follows  $c_a$  and their relative differences do not change; (c)  $c_i$  transiently rises faster than  $c_a$  so that  $c_i/c_a$  increases and  $c_a - c_i$  decreases. The control of  $c_i$  (the capacity of the plant to reduce the impact of changes in  $c_a$ , or

$c_i$  homeostasis) decreases from scenario (1) to (2) and from scenario (a) to (c) above.

Species-specific acclimations to recent increases in atmospheric  $\text{CO}_2$  concentrations were evaluated from temporal variations in the ratio and the differences between  $c_i$  and  $c_a$  (Fig. 3, Table 2). In general, the species considered in our study showed quite a similar behavior of  $c_i$  at the increasing  $c_a$  registered in the last few years, with lower  $c_i/c_a$  ratios observed in conifers compared to hardwood trees (Fig. 3a). In agreement to published studies (Guerrieri et al. 2019) this evidence could underline species-specific differences in mesophyll conductance (Flexas et al. 2008) and leaf photosynthetic components (Bahar et al. 2018). However, this increasing trend (Fig. 3b) did not directly translate in a common response of selected species when considering time-related trends, accounting for cambial age and within tree variation (Table 2). Indeed, all species (except for *Cupressus*) showed flat  $iWUE$  trends, possibly linked to variations in either  $A$  or  $g_s$ .

Our results at first confirm that not all tree species save water equally in either conifer and hardwood species (Leonardi et al. 2012; Saurer et al. 2014; Frank et al. 2015); also we must recognize that our study, although considering sites with quite similar climate variations (Figs. S1 and S2), considers artificial plantation areas of introduced tree species where site-specific conditions, in particular water



table depths, are quite different. Indeed at *Site 1*, a greater available soil water reservoir for trees is vital to mitigate the occurrence of drought stress events; conversely, at *Site 2*, conifer plantations are subjected to high temperatures and frequent drought episodes that would lower soil water potential, which can induce stomatal closure (McDowell et al. 2008) and reduce photosynthetic carbon uptake (Wang et al. 2017). At elevated temperatures, plants tend to close their stomata to prevent excessive water loss, increasing *iWUE*, as observed, for example, in pine forests on the Iberian Peninsula (Andreu-Hayles et al. 2011). Moreover, high temperature and extreme heat events can worsen drought stresses (Teskey et al. 2015) and exacerbate the effects of increasing CO<sub>2</sub> concentrations on *iWUE* (Norby and Zak 2011; Tognetti et al. 2014; Kwak et al. 2016).

In addition, the observed patterns could be partially related to differences in carbon allocation (e.g., increasing leaf area index or developing a deeper root system) among the species. Indeed, except for *Populus*, the hardwood species at *Site 1* did not show enhanced growth in terms of basal area (Fig. 1c). Therefore, there is no evidence here to support an increase in *A*; a reduction in *g<sub>s</sub>* is most likely responsible for the observed decreasing *c<sub>a</sub>-c<sub>i</sub>* patterns. In our data set, we observed constant *c<sub>i</sub>/c<sub>a</sub>* trends correlated with an increase in *c<sub>a</sub>-c<sub>i</sub>* and *iWUE* for conifers while for broadleaves, flat or slightly increasing *c<sub>i</sub>/c<sub>a</sub>* trends associated to a flat or slightly decreasing *c<sub>a</sub>-c<sub>i</sub>* and *iWUE* trends (Table 2). Our results suggest that conifers and *Cupressus* in particular, has the highest plasticity in terms of regulating stomata in response to rising atmospheric CO<sub>2</sub> concentrations; as the increase in *iWUE* was associated with constant *c<sub>i</sub>/c<sub>a</sub>*. In these specific cases, our observations support the hypothesis that there is an active mechanism maintaining a constant *c<sub>i</sub>/c<sub>a</sub>* ratio associated with increased *iWUE*, as corroborated by previous studies (Peñuelas and Azcon-Bieto 1999; Feng 1999; Saurer et al. 2004; Peñuelas et al. 2008, 2011).

## Conclusions

The following main conclusions can be drawn from our study:

1. *BAI* and *iWUE* showed a high species-specific degree of variation depicting less and more efficient species within conifers and hardwood trees; site-specific climate conditions and water availability differentially influenced *iWUE*.
2. All the considered species showed similar short-term ecophysiological adjustments with respect to the ongoing rise in CO<sub>2</sub> concentrations; in a few cases, the hypothesis of an active plant mechanism, maintaining a constant ratio between intercellular and ambient CO<sub>2</sub>

concentrations, was observed, i.e. *Cupressus*, the less productive and more water saving within the considered species.

3. *Juglans*, *Fraxinus* and *Pinus* seem to show the best performance in terms of carbon and water balance, providing functional-based indications on tree species selection for forestry plantations.

However, further studies incorporating a greater number of conifers and hardwood species are necessary; in this sense, this study can give first useful attempts.

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## Declarations

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

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