Xylem morphology determines the drought response of two *Arundo donax* ecotypes from contrasting habitats

MATTHEW HAWORTH¹, MAURO CENTRITTO¹, ALESSIO GIOVANNELLI¹, GIOVANNI MARINO¹, NOEMI PROIETTI², DONATELLA CAPITANI², ANNA DE CARLO¹ and FRANCESCO LORETO³

¹Tree and Timber Institute, National Research Council (CNR - IVALSA), Via Madonna del Piano 10 Sesto Fiorentino, 50019 Firenze, Italy, ²Institute of Chemical Methodologies, National Research Council (CNR - IMC), Area della Ricerca di Roma 1, Via Salaria Km 29,300, 00015 Monterotondo, Rome, Italy, ³Department of Biology, Agriculture and Food Sciences, National Research Council (CNR-DiSBA), Piazzale Aldo Moro 7, 00185 Roma, Italy

**Abstract**

*Arundo donax* exhibits rapid growth and requires little nutrient input, making it an ideal perennial biomass crop species. However, this growth is accompanied by high rates of water use, potentially restricting the use of *A. donax* in rain-fed marginal lands. Here, we investigated the physiological and morphological responses to drought in two ecotypes of *A. donax* from contrasting habitats: one from an arid environment in Morocco, and the second from a warm humid sub-Mediterranean climate in central Italy. Prolonged drought resulted in identical reductions in leaf-level photosynthesis (*Pₙ*ₙ) and stomatal conductance (*Gₛ*) in the two ecotypes. However, water deficit induced an increase in xylem vessel diameter in the Moroccan plants, improving the movement of water along the stem, but also likely reducing the resistance to embolism. In contrast, the Italian ecotype reduced xylem vessel area, thus increasing resistance to water transport and xylem embolism. The increased xylem vessel size and associated vulnerability to embolism in the Moroccan plants may have contributed to an increase in the loss of leaf numbers, but also to higher relative water content (RWC) in the remaining leaves in comparison to the Italian ecotype, where a greater number of leaves persisted. Despite the Moroccan plants possessing stems with a lower basal area than their Italian counterparts, both ecotypes exhibited identical leaf to supporting stem area ratios under both control and water deficit conditions. This may account for the similarities observed in leaf area measures of *Pₙ*ₙ and *Gₛ* in this and previous studies of different *A. donax* ecotypes. Selection of *A. donax* ecotypes on the basis of xylem responses to drought may facilitate the development of varieties suited to arid environments prone to severe drought and wetter habitats where prolonged droughts occur less frequently.

**Keywords:** biomass, giant reed, hydraulic conductivity, marginal lands, photosynthesis, stomatal conductance, xylem embolism

Received 17 October 2015; revised version received 27 November 2015 and accepted 5 December 2015

**Introduction**

The giant reed (*Arundo donax*) is a highly promising perennial biomass crop for low productivity marginal lands in warm to hot climates (Corno et al., 2014). *Arundo donax* exhibits rapid growth (Angelini et al., 2009), requires little nutrient input (Cosentino et al., 2014), contributes to soil stability through sequestration of large volumes of organic carbon (Kort et al., 1998; Monti & Zatta, 2009; Sarkhot et al., 2012) and produces significant amounts of extractable lignocellulosic materials required for the production of bio-ethanol and -compounds (Scordia et al., 2014; Saikia et al., 2015; Zucaro et al., 2015). As *A. donax* commonly grows in areas with high soil water content, frequently adjacent to water bodies (Danin, 2004; Coffman et al., 2010; Watts & Moore, 2011), its effectiveness as a biomass crop may be limited in marginal lands with low water availability (Shi et al., 2012; Gelfand et al., 2013; Mann et al., 2013) where supplementary irrigation is not possible due to cost, inaccessibility or scarcity of water (Grierson et al., 2011; Shortall, 2013). However, field trials in Mediterranean conditions suggest that while growth is diminished under rain-fed compared to irrigated conditions (Cosentino et al., 2014), *A. donax* retains a high degree of productivity in comparison to other potential perennial biomass crop species (Angelini et al., 2009). Nonetheless, the development of *A. donax* as a biomass crop necessitates the identification of highly productive drought tolerant ecotypes/genotypes (Cosentino et al., 2006); particularly as future climate predictions suggest...
increased frequency, severity and duration of drought events in areas suitable for *A. donax* cultivation (Sheffield & Wood, 2008; Hoerling et al., 2012).

Genetic variation between cultivars has been associated with productivity and drought resistance in both food (Monneveux et al., 2006; Centritto et al., 2009) and biomass (Souch & Stephens, 1998; Clifton-Brown & Lewandowski, 2000) crops. However, it is unclear if the attributes required for high productivity are compatible or mutually exclusive with those responsible for drought tolerance (Hurd, 1974; Manickavelu et al., 2006; Passioura, 2012; Tardieu, 2012). For example, highly productive ecotypes of *Oryza sativa* exhibit larger proportional reductions in yield during water deficit than their more drought tolerant equivalents, but critically still generate larger yields under both rain-fed and irrigated conditions (Lauteri et al., 2014). This genetic variation relies upon sexual reproduction, but the seeds of *A. donax* are sterile, and it spreads clonally by layering, rhizome extension and flood dispersal of stem and rhizome fragments (Boland, 2006; Mariani et al., 2010). Genetic analysis of *A. donax* in Europe and North America suggests that this clonal reproduction has resulted in low genetic diversity (Khudamrongswat et al., 2004; Ahmad et al., 2008; Mariani et al., 2010; Pilu et al., 2014). In contrast, expansion of *A. donax* in south-eastern Australia was accompanied by genetic variation possibly associated with mutation (Haddadchi et al., 2013). In the context of identifying varieties and/or traits desirable for production on drought prone marginal lands it is important to assess the photosynthetic responses of *A. donax* genotypes under water deficit and optimal growth conditions.

A two-year field trial of 40 *A. donax* ecotypes collected from southern Italy and initially grown with supplementary irrigation suggested a limited degree of variation between ecotypes in terms of biomass productivity (Cosentino et al., 2006). Similar differences in the biomass yield of eight *A. donax* ecotypes from Italy, Spain, and Germany grown in pots under well-watered conditions were observed; but under drought stress, all varieties produced identical levels of biomass (Sánchez et al., 2015). Yield in *A. donax* is positively related to stem density, stem weight, and plant height (Cosentino et al., 2006; Angelini et al., 2009). Analysis of molecular markers and morphology in eight *A. donax* ecotypes from Italy indicated the existence of three distinct genotypes. In the three genotypes, stem height and diameter were highly heritable traits between the parent plants and their progeny (Pilu et al., 2014). This may suggest that while genetic diversity in *A. donax* is relatively low, it may be possible to identify genotypes suited to biomass production on the basis of stem characteristics. As plant height is closely linked to the ability to transport water to the leaves this variation is likely to be associated with modification of xylem and may interact with drought tolerance (e.g., Hacke et al., 2000).

The levels of $P_N$ observed in *A. donax* (~30–40 $\mu$mol m$^{-2}$ s$^{-1}$ under saturating light and ambient [CO$_2$]) necessitate high rates of $G_s$ (Rossa et al., 1998). These high values of $P_N$ and $G_s$ can only be sustained through the effective uptake and transport of water (Santiago et al., 2004; Haworth et al., 2015) and regulation of stomatal control during episodes of water deficit (Cochard, 2002). Effective stomatal control in *A. donax* (Sánchez et al., 2015) prevents these high rates of $G_s$ leading to xylem embolism during episodes of excessive transpirative demand (Salleo et al., 2000; Maherali et al., 2004). Reduced leaf water potential associated with drought (Comstock & Mencuccini, 1998) or a failure in xylem vessel function (Cochard, 2002) can induce stomatal closure. Reduced $G_s$ may also occur prior to any reduction in leaf turgor (Trejo & Davies, 1991) due to root to shoot signals of soil drying (Wilkinson & Davies, 2002). This initial decrease in $G_s$ occurs prior to any reduction in the photosynthetic capacity for photosynthetic assimilation of carbon (Centritto et al., 2003; Flexas et al., 2013). Therefore, stomatal regulation of $P_N$ and leaf water content is likely to play a major role in the response of a fast growing species such as *A. donax*.

The high rate of carbon assimilation in *A. donax* (Rossa et al., 1998; Ahrar et al., 2015) permits rapid stem growth of ~2–5 m per year (Angelini et al., 2005; Cosentino et al., 2006, 2014). These levels of $P_N$ and $G_s$ require effective transport of water along the stem. However, tall grasses with rapid growth such as *A. donax* face a trade-off between structural support determined by stem density, efficiency of water transport and resistance to embolism (Neufeld et al., 1992; Tyree et al., 1994; Cochard, 2002). In vessel-bearing angiosperms, water moves most efficiently in xylem tissue with a large vessel area (and correspondingly lower density). However, xylem vessels with a large diameter are more susceptible to embolism than smaller vessels (Tyree & Sperry, 1989; Smith et al., 2013). Water moves through xylem tissue under negative pressure, if the structure of the vessel fails, or air enters the vessel, a xylem embolism forms. This impairs the flow of water along the stem (Sperry & Pockman, 1993; Cochard et al., 1996) and may result in the loss of photosynthetic tissue (Rood et al., 2000). While many species can refill xylem vessels with water and ‘repair’ embolism (Holbrook et al., 2001; Brodersen et al., 2010), this may lead to a degradation of water transport function over time (Borghetti et al., 1998; Hubbard et al., 1999). Stomatal closure plays an important role in reducing the occurrence of xylem embolism during drought. Indeed xylem embolism may directly induce reductions in $G_s$ through a loss...
of leaf water potential, thus preventing further embolism (Nardini & Salleo, 2000; Coch et al., 2002). Close co-ordination of xylem vessels and stomatal control (Meinzer et al., 2009) is likely to occur in a large plant such as A. donax that displays high levels of transpirative water-loss.

We investigated the response to severe drought in two A. donax ecotypes from contrasting habitats: Marrakesh, Morocco (arid pre-desert; 200 mm precipitation per annum; mean summer temperature of 30 °C) and Sesto Fiorentino, central Italy (warm sub-Mediterranean; 800 mm precipitation per annum; mean summer temperature of 23 °C). We hypothesise that the Moroccan ecotype from the more arid environment will exhibit greater drought tolerance than the Italian counterpart; and that the respective water transport structures of the ecotypes may be responsible for any differences in the maintenance of gas exchange, photosynthesis, and leaf water content during water deficit. The aim of this study were to: (i) quantify any differences in growth rate, photosynthetic and stomatal responses to water deficit between the two ecotypes; (ii) investigate the effect of xylem vessel morphology on the response of A. donax to drought and recovery from drought; (iii) identify a possible drought tolerant ecotype of A. donax, or traits that confer resistance to water deficit, that may be associated with ecotypic/genotypic differences, and; (iv) assess whether any traits related to drought tolerance are compatible with high productivity under favourable growth conditions.

Materials and methods

Plant material and growth conditions

Rhizomes of ~20 cm in length and 200 g in weight were collected from single clonal plants in fields nearby Marrakesh, Morocco and Sesto Fiorentino, Florence, Italy during the spring of 2013. Each rhizome contained at least one visible growth bud. The rhizomes were planted in 50 l pots containing Amsterdam medium (a 9:1 mix of washed sand and compost) and allowed to grow for 5 months in the CNR experimental field at Sesto Fiorentino. The plants were supplied each week with a commercial liquid fertiliser to provide nutrients at free access rates (Mahmood et al., 2015). Twenty plants of equal size were selected from each ecotype at the start of the experiment. Drought stress was achieved by withholding water from the pots. The evening before the start of the experiment, all of the pots were watered to capacity and then allowed to drain overnight. Their weight was recorded the next morning, and the pots were sealed in a white plastic bag to prevent evaporation from the pot. A Combics 1 balance with an accuracy of 1 g was used (Sartorius AG, Goettingen, Germany). The pots were weighed each day and the amount of water lost from the control plants as transpiration was replaced. The plastic bags were opened for ~30 min every day to prevent the development of anoxic conditions by allowing the exchange of gases between the pot and the atmosphere. Maximum drought stress was achieved in both ecotypes 18 days after the start of the experiment when $G_s$ values reached their lowest level and remained stable for 3 days and pot weight altered by <2%. Over the course of the 18 day drought treatment the Italian A. donax plants transpired 7.936 ± 0.414 Kg of water and the Moroccan 7.824 ± 0.737 Kg (one-way ANOVA $F_{1,14} = 0.0206; P = 0.888$) (see Fig. S2). After drought stress the plants were re-watered to pot capacity each day. Fourteen days after maximum drought stress, the plants were considered to have recovered when control and drought stressed plants exhibited identical levels of $G_s$. Four replicates were destructively sampled for each ecotype and treatment at the start, maximum stress and recovery stages of the experiment. Fresh leaf area was recorded using a LiCor Li3100C (Li-Cor, Inc., Lincoln, NE, USA). Above ground dry biomass was determined after drying stems and leaves in an oven at 60 °C for 4 days.

Gas exchange

Measurements of $P_N$, $G_s$ and the internal sub-stomatal concentration of $[\text{CO}_2]$ ($C_i$) were consistently performed on the mid-section of the second fully expanded leaf from the flag leaf using a LiCor Li6400XT fitted with a 2 cm$^2$ leaf cuvette (Li-Cor, Inc., Lincoln, NE, USA). Cuvette settings of 400 ppm $[\text{CO}_2]$, 2000 $\mu$mol m$^{-2}$ s$^{-1}$ of photosynthetically active radiation (10% blue and 90% red light) and a leaf temperature of 30 °C were used. To reduce diffusive leaks through the chamber gasket, a supplementary gasket was added and the IRGA exhaust air was fed into the interspace between the chamber and the supplementary gaskets (Rodeghiero et al., 2007). Gas exchange measurements were performed between 10:00 and 12:00 each day when the plants exhibited the highest levels of $P_N$ and $G_s$. Instantaneous water use efficiency (WUEi) was calculated as the ratio of $P_N$ to evapotranspiration ($E$) derived from the leaf gas exchange measurements.

Leaf water content

The relative water content (RWC) of leaves from the A. donax ecotypes was sampled at all stages of the experiment. The second fully expanded leaf from the flag leaf was removed from four plants for each ecotype and treatment and the RWC determined using the approach of Diaz-Pérez et al. (1995).

Xylem vessel anatomy

Sections of stem 2–3 cm in length were cut from the new growth of four stems per ecotype for each treatment. These portions of the stem were then placed in a 50:50 mixture of ethanol and water and stored at 5 °C. The stem sections were then fixed through ice on a Peltier plate, and transverse sections of 8–12 μm thickness cut using a rotary microtome. The sections were stained with a solution of 0.04% safranin, 0.15% astra blue and 2% acetic acid in distilled water (Emili et al., 2011), and permanently fixed with a histological mounting.
medium (Eukitt, Bioptica, Milan, Italy) to observe the developing tissues. A Nikon Eclipse 800E light microscope connected to a Nikon DS-Fi2 microscope camera (Nikon Corporation, Tokyo, Japan) was used for anatomical observations. Digital images of the cross-sections were then analysed and transversal stem structure examination was performed on four to six independent images per sector using the computer program NIKON NS-ELEMENTS software. For each image the following parameters were measured: mean area of fascicular bundles (AF, mm²), fascicular bundles density (Fb, N mm⁻³), mean vessel diameter (Dv, mm²), and annular vessel lumen diameter (Dav, mm²). Example images of the xylem vessels of the Moroccan and Italian A. donax plants are given in Fig. S1. The diameter of each vessel was calculated as the diameter of a circle with an area equivalent to the lumen cross-section. The frequency distribution of xylem vessels was then categorised by 10 μm diameter classes. The hydraulic weighted vessel diameter DH was calculated following Sperry & Saliendra (1994) as:

\[ D_H = 2 \sum \frac{r^4}{\sum r^4} \]  

where \( r \) = radius in μm. Calculation of \( D_H \) incorporates the disproportionate contribution of large vessels to total flow and gives the average diameter needed for a given vessel density to result in the theoretical hydraulic conductivity for that stem Tyree et al. (2002). The theoretical specific xylem hydraulic conductivity (Kst) was calculated following Santiago et al. (2004) using the Hagen-Poiseuille equation for ideal capillaries assuming laminar flow:

\[ Kst = \left( \frac{\pi \rho}{128 \eta A_{\text{image}}} \right) \sum D_v^4 \]  

where \( \rho \) is the density of water (998.2 Kg m⁻³ at 20 °C); \( \eta \) is the viscosity of water (1.002 \times 10⁻³ MPa s at 20 °C), and; \( A_{\text{image}} \) is the area of the image analysed (m²).

**Stem traits**

When plants were destructively harvested for leaf area and biomass measurements, 1–2 cm long discs were cut from the stem at the 2nd, 5th, and 15th internodes from the collar. The fresh weight of the samples was immediately recorded (Green Weight, GW), and the fresh volume (Green Volume, GV) was measured by water displacement (Berta et al., 2010). The dry weight (Dry Weight, DW) was determined after drying in an oven at 103 °C for 4 days. Basic Density (BD, kg m⁻³), Green Density (GD, kg m⁻³), and the Moisture Content (MC, %) of the stems were calculated as:

\[ \text{BD} = \frac{\text{DW}}{\text{GV}} \]  
\[ \text{GD} = \frac{\text{GW}}{\text{GV}} \]  

where \( \text{MC} = \frac{(\text{GW} - \text{DW})}{\text{DW}} \times 100 \) (5)

The volumetric fractions occupied by cell wall (Wood Sub- stance, WS), Bound Water (BW), Free Water (FW), and Water Vapor Air (WVA) were determined according to the procedure of Carriero et al. (2015) assuming the following standard densities: 1500 kg m⁻³ for dry wood, 1000 kg m⁻³ for free water, and 1018 kg m⁻³ for bound water. Volumetric fractions (WS) and fibre saturation point (FSP) were calculated as:

\[ \text{WS} = \left( \frac{\text{BD}}{1500} \right) \times 100 \]  
\[ \text{BW} = \left( \frac{\text{BD}}{1018} \right) \times 30 \]  
\[ \text{FW} = \left( \frac{\text{BD}/1000}{\text{MC} - 30} \right) \]  
\[ \text{WVA} = 100 - (\text{WS} + \text{BW} + \text{FW}) \]  
\[ \text{FSP} = \left( \frac{\text{FW}}{\text{FW} + \text{WVA}} \right) \times 100 \]  

**Statistical analyses**

Statistical analyses were performed using SPSS 20 (IBM, Armonk, NY, USA). To test the effect of water deficit on the Italian and Moroccan A. donax we used one- and two-way ANO-VAs to assess differences in variance between samples associated with either ecotype or treatment effects.

**Results**

The Moroccan and Italian ecotypes of A. donax exhibited identical high levels of \( P_N \) (−30 μmol m⁻² s⁻¹) and \( G_s \) (0.4–0.6 mol m⁻² s⁻¹) under control conditions (Fig. 1a, b). These values are consistent with previously published gas exchange values of A. donax under field conditions (Rossa et al., 1998). Severe drought reduced both \( G_s \) and \( P_N \) similarly in both genotypes (>90% reduction in both parameters) (Fig. 1a, b). However, \( C_i \) was lower in the Moroccan ecotype (Fig. 1c). Rates of \( P_N \) and \( G_s \) in the recovery period were lower in both ecotypes than those recorded in the control plants at the start and maximum stress stages of the study as temperatures were slightly cooler in the final weeks of the experiment.

The volume of water used (Fig. 2a) and the water use efficiency (WUE), gauged as the amount of whole plant biomass change relative to water use (Fig. 3b) were
identical in both A. donax ectypes under control and drought stress conditions. However, WUEi measured using leaf-level gas exchange at maximum drought stress was 30.8% higher in the Moroccan ecotype than in the Italian counterpart (Fig. 2c). Mean plant biomass yield was, respectively, reduced by 16.1 and 6.3% in drought stressed Moroccan and Italian A. donax. This loss occurred with 55.6 and 68.1% reductions in leaf area in the Moroccan and Italian ecotypes. The lower proportional leaf loss in the Moroccan plants may be associated with its lower leaf area at the start of the study, despite no significant difference being observed in total leaf area between the two ecotypes (Table 1). No significant difference was found in leaf number between the A. donax ecotypes at the start of the study or at maximum drought stress; however, the Moroccan lost a higher proportion (53.8%) of leaves than the Italian (38.1%) ecotype. No statistical difference was observed between the number of stems per plant or stem height between ecotypes or treatment (Table 1). The Moroccan A. donax ecotype maintained a higher leaf RWC than the Italian ecotype during drought stress. Relative water content was reduced from 92–93.6% to 80.2% in the Moroccan and 59.1% in the Italian ecotype (Fig. 2d).

Analysis of the A. donax xylem vessels collected from the most recent stem growth at the maximum stress and recovery stages of the study suggest differences in xylem traits between the two ecotypes and in their response to water deficit (Table 2 and Fig. 3). The Italian ecotype exhibited lower xylem vessel lumen area and total conducting area than the Moroccan plants under control conditions. Furthermore, withholding of water resulted in contrasting responses in the two ecotypes: the Moroccan plants increased mean bundle area, mean vessel area, and total conducting area, while these parameters all declined in the Italian A. donax (Table 2). At maximum drought stress a higher proportion of xylem vessels occurred in smaller diameter size classes (0–40 μm) in the Italian ecotype. In contrast, the Moroccan plants showed a higher frequency of larger xylem vessels (80–110 μm diameter) (Fig. 3). Moreover, drought stress increased the proportion of large xylem vessels, and reduced the proportion of xylem vessels around the median in the Moroccan plants, resulting in a more even distribution of xylem vessel size classes than in the Italian A. donax (Table 2 and Fig. 3). This effect becomes more apparent in the Moroccan plants during the recovery phase, where the proportion of smaller xylem vessels falls further in the drought stressed plants, which exhibit proportionally more vessels in the larger xylem vessel size classes. A similar pattern was observed in the Italian plants during the recovery stage, where the drought stressed plants increased the proportion of xylem vessels in comparison to the control plants; however, this occurred in lower xylem vessel diameter classes than in the Moroccan ecotypes (60–80 μm in the Italian, and 80–110 μm in the Moroccan) (Fig. 3).

The greater abundance of larger xylem vessels in the Moroccan ecotype will disproportionately contribute to total water flow. Therefore, DH was calculated as the average vessel diameter required for a set vessel density to result in the theoretical hydraulic conductivity for that stem (Kst) (Fig. 4). This approach permits analysis
of the water transport properties of the stem independent of variations in stem density. In contrast to the actual measures of vessel size, where no significant difference was observed (Table 2), calculation of DH suggests that drought stress induced a statistically significant increase in vessel diameter in the Moroccan ecotype, but not in the Italian ecotype, where no significant alteration in DH occurred (Fig. 7a). The larger xylem vessels that developed in the Moroccan drought stressed plants induced an increase in Kst of the xylem tissue at both the maximum drought stress and recovery stages of the experiment (Fig. 4b).

Despite the apparent differences in water transport capacity (Figs 6 and 7), the two ecotypes exhibited remarkably similar leaf-level rates of $P_N$ and $G_s$ under control and drought conditions (Fig. 1). Significant differences were not observed in mean stem height or stem number per plant between the ecotypes or treatments (Table 1). However, the cross-sectional area of the stems in the Moroccan plants was lower than in the Italian $A. \text{donax}$ (Table 1); this did not correspond to a difference in stem density between the ecotypes (Table 3). The number of leaves lost during drought stress was greater in the Moroccan ecotype (Table 1). However, the Italian ecotype possessed leaves with a greater area and predominantly lost it largest leaves, whereas the Moroccan $A. \text{donax}$ shed leaves more evenly (Fig. 5a). These differences in leaf loss and stem area in response to drought between the two ecotypes may account for the similarity in the ratios of stem to leaf area (Fig. 5b). The Huber value (or AL/AS ratio) gives an indication of the water consumption relative to
under control conditions both of the ecotypes display Huber values of $\sim 0.0006 \text{ m}^2 \text{ cm}^{-2}$, and under drought stress this increases three-fold to $\sim 0.0018 \text{ m}^2 \text{ cm}^{-2}$. We observed a significant reduction in stem density under water deficit in both A. donax ecotypes.

**Table 1** Mean value and standard error for morphological traits measured in the Italian and Moroccan Arundo donax ecotypes and water treatment at the maximum drought stress stage of the study

| Ecotype | Treatment | Mean stem Height (cm) | Number of stems per plant | Stem Basal area (mm$^2$) | Number of leaves | Total leaf area (cm$^2$) | Mean Biomass (g) (DW) |
|---------|-----------|-----------------------|---------------------------|--------------------------|------------------|------------------------|-----------------------|
| Italian | Control   | 79.0 ± 11.5$^a$       | 4.9 ± 1.8$^a$             | 45.1 ± 4.0$^a$           | 67.0 ± 13.7$^a$ | 5130.3 ± 1528.4$^a$   | 102 ± 10.9$^a$       |
|         | Drought   | 84.8 ± 11.1$^a$       | 4.7 ± 2.1$^a$             | 45.9 ± 3.9$^a$           | 41.5 ± 7.7$^b$  | 1692.3 ± 347.1$^b$    | 99.4 ± 4.6$^a$       |
| Moroccan| Control   | 74.8 ± 4.4$^a$        | 5.8 ± 1.6$^a$             | 30.1 ± 1.7$^b$           | 60.8 ± 7.4$^a$  | 3724.9 ± 1176.1$^a$   | 80.4 ± 11.2$^b$      |
|         | Drought   | 81.5 ± 18$^a$         | 5.6 ± 2.9$^b$             | 33.2 ± 3.8$^b$           | 28.1 ± 3.2$^b$  | 1654.2 ± 763.1$^b$    | 85.4 ± 6.6$^b$       |

Two-way ANOVA

|                      | Ecotype | Treatment | $F_{1,14}$ values | $P$ | $P$ values | $P$ values | $P$ values | $P$ values |
|----------------------|---------|-----------|-------------------|-----|------------|------------|------------|------------|
|                      |         |           |                   |     | 0.64 ns    | 1.3 ns     | 14.1***    | 1.2 ns     |
|                      |         |           |                   |     | 1.81 ns    | 0.08 ns    | 0.29 ns    | 11.2***    |
|                      | Ecotype | x         | 0.08 ns           | 0.002 ns | 0.09 ns | 1.9 ns | 0.0 ns | 0.6 ns |
|                      | Treatment |           |                   |     | 1.92 ns    | 0.0 ns    | 1.2 ns    | 0.0 ns    |

A two-way ANOVA was used to assess any significant differences between treatment and/or genotype. Different letters indicate significant differences between datasets based upon a one-way ANOVA with LSD post hoc test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

Fig. 3 Frequency distribution of xylem vessel diameter size classes of Moroccan and Italian Arundo donax ecotypes at maximum stress and recovery stages of the study. Error bars indicate one standard deviation either side of the mean.
Different A two-way ANOVA was used to assess any significant differences between treatment and/or genotype. Different letters indicate significant differences between datasets based upon a one-way ANOVA with LSD test.

**Table 2** Mean value and standard error for xylem vessel traits measured in the Italian and Moroccan *Arundo donax* ecotypes and water treatment at the maximum drought stress stage of the study

| Ecotype       | Treatment | Xylem bundles density (n⁶ mm⁻²) | Mean area of xylem bundle (µm²) | Xylem vessel lumen (µm²) | Xylem lumen Annular Vessel (µm²) | Conducting area (µm²) |
|---------------|-----------|---------------------------------|---------------------------------|-------------------------|---------------------------------|-----------------------|
| Italian       | Control   | 4.4 ± 0.6⁴                     | 56859.4 ± 4319.1⁴               | 3902.3 ± 207.3¹         | 965.3 ± 76.9¹                   | 9900.4 ± 685.2⁴       |
|               | Drought   | 4.8 ± 0.1¹                     | 52582.1 ± 2965.3³               | 3550.1 ± 189.5²         | 833.9 ± 82.0³                   | 8823.6 ± 591.1³       |
| Moroccan      | Control   | 4.6 ± 0.3¹                     | 60005.8 ± 2342.8⁴               | 4020.1 ± 144.7¹         | 1018.7 ± 73.8¹                  | 10077.5 ± 347.8²      |
|               | Drought   | 4.8 ± 0.8³                     | 62880.1 ± 2534.6⁴               | 4565.8 ± 152.4³         | 1112.9 ± 106.2³                 | 11209.2 ± 584.5³      |

Two-way ANOVA

| Ecotype       | Treatment | F₁₁,₁₄ values | Mean | Xylem vessel lumen | Xylem lumen | Conducting area |
|---------------|-----------|---------------|------|-------------------|-------------|----------------|
|               |           |               | 0.01 | 3.3 | 8.32** | 3.4 | 4.9* |
| Treatment     |           |               | 0.24 | 0.03 | 0.24  | 0.04 | 0.1n |
| Ecotype x     |           |               | 0.02 | 0.93 | 5.2* | 1.6 | 2.1n |
| Treatment     |           |               | 0.6a | 56859.4 | 3.4 | 5.2 | 1.6 |

Different A two-way ANOVA was used to assess any significant differences between treatment and/or genotype. Different letters indicate significant differences between datasets based upon a one-way ANOVA with LSD post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

Fig. 4 (a) The average vessel diameter required for a set vessel density to result in the theoretical hydraulic conductivity (D₃₅) for that stem. (b) theoretical hydraulic conductivity for the stem (Kₘₙ). Error bars indicate one standard error either side of the mean. Different letters indicate significant difference using a one-way ANOVA and LSD post hoc test.

**Discussion**

The Moroccan and Italian *A. donax* ecotypes exhibited identical leaf-level photosynthetic and stomatal responses to drought stress (Fig. 1). This would appear to be consistent with previous studies suggesting low genetic diversity in *A. donax* (Ahmad et al., 2008; Mariani et al., 2010; Pilu et al., 2014), and observations of broadly consistent gas exchange responses under water deficit conditions (Sánchez et al., 2015). However, the two ecotypes studied showed contrasting responses to drought stress in terms of the modification of xylem vessel morphology (Table 2 and Fig. 3) and leaf loss (Table 1 and Fig. 5). This may suggest that the *A. donax* plants have adapted to the respective climatic conditions and common abiotic stresses associated with growth in arid (Moroccan) and warm humid sub-Mediterranean (Italian) habitats.

**Leaf gas exchange of Arundo donax**

*Arundo donax* exhibits extremely high rates of carbon assimilation (Rossa et al., 1998; Nackley et al., 2014; Ahrar et al., 2015). However, this PSₙ is accompanied by high rates of water-loss (Fig. 1b) (Rossa et al., 1998; Watts & Moore, 2011), potentially making *A. donax* vulnerable to drought stress (e.g., Manickavelu et al., 2006). Severe drought stress induced stomatal closure in the two ecotypes (Fig. 1b). The Moroccan ecotype also exhibited lower Cᵢ levels at the maximum drought stress stage, possibly suggesting that carbon recycling was still occurring (Scheuermann et al., 1991), or that the degree of drought stress was less severe than in the Italian ecotype. Stomatal closure generally accompanies water deficit to reduce transpirative water-loss (e.g., Lauteri et al., 2014), the initial stages of stomatal closure are accompanied by a reduction in Cᵢ, and then when the drought stress becomes more severe and photochemistry becomes impaired, Cᵢ rises (Flexas et al., 2002). Stomatal conductance and pot weight values in our study had remained broadly consistent for
3 days in both ecotypes, suggesting that maximum drought stress had been achieved. The slightly higher values of \(G_s\) and lower values of \(C_i\) (Fig. 1) at maximum drought stress may be linked to the ability to maintain higher foliar water content of the remaining upper leaves in the Moroccan \(A.\ donax\) (Fig. 2d). Maintenance of leaf water content is fundamental to sustaining photochemistry during drought stress (Ritchie et al., 1990; Thomas & Eamus, 1999). The greater leaf water content of the Moroccan plants may be due to a greater loss of leaf numbers than in the Italian ecotype (Table 1), permitting upkeep of RWC in the younger leaves (Fig. 4). Sánchez et al. (2015) observed rates of leaf loss varying from 37.4 to 69.1% in eight \(A.\ donax\) ecotypes exposed to water deficit, suggesting that leaf loss may be a source of variation that may be exploited in the development of \(A.\ donax\) cultivars tolerant to drought.

**Xylem traits and leaf water content**

Water transport via xylem vessels is integral to the maintenance of leaf gas exchange and a stable leaf RWC (Cochard, 2002; Gleason et al., 2012). The development

### Table 3  Mean value and standard error for stem properties measured in the Italian and Moroccan Arundo donax ecotypes and water treatment at the maximum drought stress stage of the study

| Ecotype     | Treatment  | BD (Kg m\(^{-3}\)) | GD (Kg m\(^{-3}\)) | BW (%)  | MC (%)  | FW (%)  | FSP (%) |
|-------------|------------|---------------------|---------------------|---------|---------|---------|---------|
| Italian     | Control    | 528.0 ± 38.4\(^{a}\) | 1154.2 ± 17.2\(^{a}\) | 15.6 ± 1.1\(^{a}\) | 126.3 ± 15.7\(^{a}\) | 46.8 ± 3.5\(^{a}\) | 95.1 ± 3.4\(^{a}\) |
| Moroccan    | Control    | 576.6 ± 37.4\(^{a}\) | 1130.8 ± 27.8\(^{a}\) | 17 ± 1.1\(^{a}\)   | 98.1 ± 11.0\(^{a}\)  | 38.1 ± 4.5\(^{a}\)  | 85 ± 5.7\(^{a}\)  |

#### Two-way ANOVA

| Ecotype     | Treatment  | \(F_{1,14}\) values | BD          | GD          | BW          | MC          | FW          | FSP          |
|-------------|------------|----------------------|-------------|-------------|-------------|-------------|-------------|--------------|
| Italian     | Control    | 0.49ns               | 0.15ns      | 0.51ns      | 0.58ns      | 1.2ns       | 2.4ns       |              |
| Moroccan    | Control    | 10.4**               | 4.5*        | 10.3**      | 7.9**       | 8**         | 0.14ns      |              |

BD = basic density; GD = green density; BW = bound water; MC = moisture content; FW = free water; FSP = fibre saturation point. Calculations for each parameter are given in Materials and Methods. A **two-way ANOVA** was used to assess any significant differences between treatment and/or genotype. Different letters indicate significant differences between datasets based upon a **one-way ANOVA** with LSD post hoc test. \(*P < 0.05, **P < 0.01, ***P < 0.001\) and ****P < 0.0001.

**Fig. 5** (a) Box-plot representing the range of the area of individual leaves in Moroccan and Italian Arundo donax ecotypes at the start of the study and at maximum drought stress. The box signifies the distribution of the 25–75% quartiles, the median is represented by a vertical line within the box, the black square indicates the mean value, horizontal bars indicate minimum/maximum values and circles symbolise outlying data points. (b) The ratio of stem to leaf area (Huber value) in the Moroccan and Italian Arundo donax ecotypes at the maximum drought stress stage of the study. Different letters indicate significant differences between datasets based upon one-way ANOVA and LSD post hoc test.
of woody tissue represents a trade-off between structural support, the ability to move water efficiently and resistance to xylem embolism. This is reflected in the investment in water transport tissue and is imposed by the growth conditions of the plant (Lovisolo & Schubert, 1998). Drought stress exerts a number of pressures on water transport through excessive transpirative demand and constrained water uptake, which become apparent in the occurrence of xylem embolism events (Hacke et al., 2001). Under control conditions with no water limitations, both A. donax ecotypes exhibit similar xylem vessel areas (Table 2), but the Moroccan has proportionally fewer small and a greater number of larger vessels (Fig. 3). However, under conditions of water deficit the Moroccan plants exhibit an increase in mean vessel area, while the Italian ecotype reduced xylem vessel size (Table 2 and Fig. 3). This contrasting response in terms of xylem development becomes more apparent when D4 and Kst values of the xylem tissue of the ecotypes are calculated (Fig. 4). At first appearance, this result appears somewhat counterintuitive, as smaller xylem vessels are generally considered to be more resistant to embolism under excessive transpirative demand (Tyree & Sperry, 1989) and more commonly occur in plant species exposed to water deficit that exhibit adaptation to drought stress. Conversely, larger xylem vessels permit movement of water with lower resistance, and commonly occur in plants from habitats with high water availability (Villar-Salvador et al., 1997; Hacke et al., 2000). It may therefore be expected that the Moroccan plants from the more arid habitat would not only possess smaller xylem vessels than their Italian counterparts, but would also reduce vessel diameter under water deficit (e.g., Lovisolo & Schubert, 1998; Bacelar et al., 2007). However, the increase in xylem vessel size and Kst exhibited in the Moroccan plants would facilitate the movement of water up the stem, possibly accounting for the higher leaf water content in the remaining leaves of the Moroccan plants (Fig. 2d). A. donax generally occurs next to water bodies with high soil water levels, in such locations severe drought events may be relatively infrequent and short-lived. Many plants are able to 'repair' xylem embolisms by refilling the xylem vessels with water (Cochard, 2002; Brodersen et al., 2010). This may allow A. donax to regain xylem function rapidly when water becomes more available. This may also account for the increase in xylem vessel size in the Moroccan ecotype during the maximum drought stress and recovery stages as a means of more fully exploiting any amelioration in growth conditions. However, if selective pressures had exerted this response in the Moroccan plant it may be expected that the Italian A. donax from a more humid habitat would display an identical response.

The larger xylem vessels that develop in the Moroccan plant may play a role in adaptation to more arid environments. Arundo donax responds to severe stress by losing above ground tissue and sequestering carbohydrates and nutrients in the rhizomes below ground until growth conditions become more favourable (Di Nasso et al., 2013; Mann et al., 2013). Xylem embolism has been suggested as a mechanism to induce stomatal closure via a reduction in stem and leaf water potentials (Cochard, 2002; Cochard et al., 2002). Such a mechanism may account for the more pronounced leaf loss observed in the Moroccan plants. Xylem embolism may function as part of the drought response of the Moroccan A. donax, where drought is likely to be more severe and prolonged, by reducing the amount of above ground tissue where water-loss can occur (Zimmermann, 1983; Tyree & Sperry, 1988; Cochard, 2002; Cochard et al., 2002) and thus preserving the viability of the rhizome (Mann et al., 2013). The reduction in the xylem vessel diameter in the Italian A. donax will reduce the likelihood of xylem embolism, but also increase resistance to the movement of water (Lovisolo & Schubert, 1998). This may enable the Italian ecotype to retain a larger proportion of leaves than the Moroccan A. donax (Table 1). As the Italian ecotype occurs in a more humid environment where the probability of a severe and extended drought is lower than in Morocco, this would permit the preservation of a larger proportion of the above ground biomass in the event of an improvement in conditions, and thus afford greater protection to the investment in above ground biomass.

Selection of traits conferring drought tolerance in Arundo donax

Arundo donax frequently occurs adjacent to water bodies, but this likely reflects the most common method of dispersal (Boland, 2006; Coffman et al., 2010; Mariani et al., 2010), rather than a requirement for high availability of water, as A. donax grows effectively in arid environments under rain-fed conditions (e.g., Angelini et al., 2005; Cosentino et al., 2014). Nonetheless, not only does A. donax display comparatively low genetic diversity (Khudamrongsawat et al., 2004; Ahmad et al., 2008; Mariani et al., 2010) but also the similarities between the habitats of the A. donax ecotypes may have contributed towards the identical leaf gas exchange responses observed in this (Fig 1) and other studies (Sánchez et al., 2015). Nevertheless, differences in the hydraulic architecture were observed between the Italian and Moroccan plants. This suggests that a degree of variation does exist between the ecotypes in their response to drought, but that this variation has developed within, and been constrained by, the comparatively low genetic diversity displayed by A. donax in the Mediterranean (e.g., Pilu et al., 2014).
The Moroccan plants possessed smaller basal stem areas that supported lower leaf areas than their Italian counterparts (Table 1 and Fig. 8b). Nevertheless, both ecotypes exhibited identical Huber values, suggesting little variation in the area of leaf tissue or their supporting stems. Leaves with a greater hydraulic conductivity generally exhibit higher rates of $P_N$ and $G_s$ than those with lower rates of water supply (Brodribb & Feild, 2000). This consistency in Huber values may account for the similarity in leaf-level gas exchange parameters observed between $A. \text{ donax}$ ecotypes under well-watered and water deficit conditions (Fig. 1) (Sánchez et al., 2015).

The two $A. \text{ donax}$ ecotypes analysed in this study did show variation in xylem vessel traits (Table 2) and the degree of leaf loss (Table 1 and Fig. 5a) during severe drought. The larger xylem vessels that developed during water deficit in the Moroccan ecotype may accelerate the loss of leaf tissue (Zimmermann, 1983; Tyree & Sperry, 1988) thus ensuring the persistence of the rhizome (Mann et al., 2013), a particularly important attribute in a more arid climate. The reduction in xylem vessel diameter in the Italian $A. \text{ donax}$ will increase resistance to embolism and permit the plants to retain a larger number of leaves (Tyree & Sperry, 1989). Both strategies may be suited to their respective environments, where selective pressures may favour the rapid senescence of above ground tissue in habitats where the likelihood of prolonged and severe drought is comparatively high, or in more humid habitats the preservation of above ground photosynthetic tissue to more fully exploit any amelioration in conditions. In terms of the cultivation of $A. \text{ donax}$ for biomass production on marginal lands without irrigation, selection on the basis of xylem morphology and stem attributes may permit the identification of ecotypes/genotypes suited to the prevailing conditions. For example, ecotypes/genotypes that increase xylem vessel size may be preferable in arid areas as they would facilitate the survival of the rhizome, thus ensuring a crop in subsequent years. However, this would impact upon the harvest in the year affected by drought. In habitats where drought is likely to be less severe, or the crop can receive supplementary irrigation, an ecotypes/genotype that increases resistance to xylem embolism and retain leaf area would be preferable. Analysis of further $A. \text{ donax}$ specimens from diverse geographical and ecological ranges may identify ecotypes/genotypes with contrasting leaf-level rates of $P_N$ and $G_s$ to facilitate the development of $A. \text{ donax}$ as a viable biomass crop for growth in low input marginal lands.

Conclusions

Analysis of the response to drought in two ecotypes of $A. \text{ donax}$ from arid (Moroccan) and warm sub-Mediterranean (Italian) habitats suggested a degree of variability associated with adaptation to their respective environments. Both ecotypes exhibited remarkably similar leaf-level rates of $P_N$ and $G_s$ under control and water deficit treatments (Fig. 1). Mean xylem vessel size was statistically similar between the ecotypes under well-watered conditions (Table 2), but the Moroccan plants possessed fewer xylem vessels in the smaller diameter classes (Fig. 3). As drought progressed, the Moroccan ecotype developed larger xylem vessels, while its Italian counterparts produced vessels with a lower area. The larger xylem vessels of the Moroccan plants exhibited not only greater Kst (Fig. 4b) but also a probable lower resistance to xylem embolism; potentially contributing to increased loss of leaf numbers (Table 1) but higher foliar water content in the remaining upper leaves (Fig. 2d). Increased vulnerability to xylem embolism may play a part in the drought response of $A. \text{ donax}$ in more arid habitats through accelerating the loss of above ground biomass and preserving the viability of the rhizome (e.g., Mann et al., 2013). Despite this apparent difference in xylem vessel traits, both ecotypes demonstrated identical relationships between the amount of leaf tissue supported by stem area under both control and drought stressed conditions (Fig. 5b). This similarity in Huber values between the Moroccan and Italian ecotypes may reflect low genetic diversity in $A. \text{ donax}$ (Pilu et al., 2014) and may account for observations of identical gas exchange parameters when measured on a leaf area basis.

Acknowledgements

We thank Said Wahbi (Université Cadi Ayyad, Marrakech) for providing rhizomes. We are grateful to Monica Anichini (CNR – IVALSA) for analysis of xylem vessel morphology. Marco Michelozzi and Gabriele Cencetti (CNR – IBBR) are thanked for assistance during the experiment. The comments of three anonymous reviewers significantly improved this manuscript. This study was funded by the EU FP7 project WATBIO (Development of improved perennial non-food biomass and bioproduct crops for water stressed environments – 311929) and the Ministero dell’Istruzione dell’Università e della Ricerca di Italy projects PRIN 2010–2011 PRO-ROOT and Progetto Premiale 2012 Aqua.

References

Ahmad R, Liow P-S, Spencer DF, Jasieniuk M (2008) Molecular evidence for a single genetic clone of invasive Arundo donax in the United States. Aquatic Botany, 88, 113–120.

Ahzar M, Doneva D, Koleva D et al. (2015) Isoprene emission in the monocot Arundo donax L. (Poaceae) tribe in relation to functional and structural organization of the photosynthetic apparatus. Environmental and Experimental Botany, 119, 87–95.

Angelini L, Cecutarini L, Bonari E (2005) Biomass yield and energy balance of giant reed (Arundo donax L.) cropped in central Italy as related to different management practices. European Journal of Agronomy, 22, 375–389.
Neufeld HS, Grantz DA, Meinerz FC, Goldstein G, Crisosto GM, Crisosto C (1992) Genotypic variability in vulnerability of leaf xylem to cavitation in water-stressed and well-irrigated sugarcane. *Plant Physiology*, **100**, 1020–1028.

Passioura J (2012) Phenotyping for drought tolerance in grain crops: when is it useful to breeders? *Functional Plant Biology*, **39**, 851–859.

Pita R, Cassani E, Landoni M et al. (2014) Genetic characterization of an Italian giant reed (*Arundo donax L.*) clones collection: exploiting clonal selection. *Euphytica*, **196**, 169–181.

Ritchie SW, Nguyen HT, Holaday AS (1990) Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. *Crop Science*, **30**, 105–111.

Rodeghiero M, Ninnemets Ü, Cescatti A (2007) Major diffusion leaks of clamp-on leaf cuvettes still unaccounted: how erroneous are the estimates of Farquhar et al. model parameters? *Plant, Cell & Environment*, **30**, 1006–1022.

Rood SB, Patiño S, Cooks K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees*, **14**, 248–257.

Rossa B, Tüffer A, Naidoo G, Willett D (1998) *Arundo donax* L. (Poaceae)—a C3 species with unusually high photosynthetic capacity. *Botanica Acta*, **111**, 216–221.

Sakka R, Chutia RS, Kataki R, Pant KK (2015) Perennial grass (*Arundo donax* L.) as a feedstock for thermo-chemical conversion to energy and materials. *Bioresource Technology*, **188**, 265–272.

Salleo S, Nardini A, Pitt F, Guillo MAL (2000) Xylem cavitation and hydraulic control of stomatal conductance in laurel (*Laurus nobilis* L.). *Plant, Cell and Environment*, **23**, 71–79.

Sánchez E, Scordia D, Lino G, Arias C, Cosentino S, Nogués S (2015) Salinity and water stress effects on biomass production in different *Arundo donax* L. clones. *Bioenergy Research*, **8**, 1461–1479.

Santiago L, Goldstein G, Meinerz F, Fisher J, Machado K, Woodruff D, Jones T (2004) Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia*, **140**, 543–550.

Sarkhot DV, Grunwald S, Ge Y, Morgan CLS (2012) Total and available soil carbon fractions under the perennial grass *Cynodon dactylon* (L.) Pers and the bioenergy crop *Arundo donax* L. *Biomassa and Bioenergy*, **41**, 122–130.

Scheuermann R, Beheker K, Stühlthauft F, Fock H (1991) Simultaneous gas exchange and fluorescence measurements indicate differences in the response of sunflower, bean and maize to water stress. *Photosynthesis Research*, **27**, 189–197.

Scordia D, Testa G, Cosentino SL (2014) Perennial grasses as lignocellulosic feedstock for second-generation bioethanol production in Mediterranean environment. *Italian Journal of Agronomy*, **9**, 84–92.

Sheffield J, Wood EF (2008) Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, IPCC AR4 simulations. *Climate Dynamics*, **31**, 79–105.

Shi Q, Cao Y, Hu H (2012) Effects of drought stress on stalk germination and seedling growth of *Arundo donax*. *Research of Soil and Water Conservation*, **2**, 1–45.

Shortall O (2013) “Marginal land” for energy crops: exploring definitions and embedded assumptions. *Energy Policy*, **62**, 19–27.

Smith MS, Fridley JD, Yin J, Bauerle TL (2013) Contrasting xylem vessel constraints on hydraulic conductivity between native and non-native woody understory species. *Frontiers in Plant Science*, **4**, 1–12.

Souch C, Stephens W (1998) Growth, productivity and water use in three hybrid poplar clones. *Tree physiology*, **18**, 829–835.

Sperry J, Fockman W (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell & Environment*, **16**, 279–287.

Sperry J, Salendrea N (1994) Intra-and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment*, **17**, 1233–1241.

Tardieu F (2012) Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. *Journal of Experimental Botany*, **63**, 25–31.

Thomas DS, Eamus D (1999) The influence of predawn leaf water potential on stomatal responses to atmospheric water content at constant C3 and on stem hydraulic conductance and foliar ABA concentrations. *Journal of Experimental Botany*, **50**, 243–251.

Trejo C, Davies WJ (1991) Drought-induced closure of *Phaseolus vulgaris* L. stomata precedes leaf water deficit and any increase in xylem ABA concentration. *Journal of Experimental Botany*, **42**, 1507–1516.

Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress?: answers from a model. *Plant Physiology*, **88**, 574–580.

Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Biology*, **40**, 19–36.

Tyree MT, Davis SD, Cochard H (1994) Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *IAWA Journal*, **15**, 335–360.

Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three *Quercus* (Fagaceae) species along a climatic gradient in NE Spain. *Trees*, **12**, 90–96.

Watts DA, Moore GW (2011) Water-use dynamics of an invasive reed, *Arundo donax*, from leaf to stand. *Wetlands*, **31**, 725–734.

Wilkinson S, Davies WJ (2002) ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, Cell and Environment*, **25**, 195–218.

Zimmermann MH (1983) *Xylem Structure and the Ascent of Sap*. Springer, New York.

Zucaro A, Forte A, Fagnano M, Bastianoni S, Basosi R, Fierro A (2015) Comparative attributional life cycle assessment of annual and perennial lignocellulosic feedstocks production under mediterranean climate for biorefinery framework. *Integrated Environmental Assessment and Management*, **11**, 397–403.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Representative images of xylem structures in the *Arundo donax* plants studied in this experiment.

**Fig. S2.** Daily water-loss in Italian and Moroccan *Arundo donax* plants measured gravimetrically.

**Fig. S3.** Photosynthesis (Pn) and stomatal conductance (Gs) in Moroccan and Italian *Arundo donax* ecotypes subjected to severe drought (linear regression, $F = 396.803; P = 1.482 \times 10^{-30}$).

**Fig. S4.** Relative water content (RWC) at all stages of the drought and recovery treatments, and non-destructive measurement using nuclear magnetic resonance (NMR) at the maximum stage of the drought stress on the same leaf used for gas exchange and subsequently for determination of RWC.