GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2015) 7, 811–819, doi: 10.1111/gcbb.12172

Evapotranspiration, crop coefficient and water use efficiency of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus × giganteus* Greef et Deu.) in a Mediterranean environment.

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Abstract

Giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus × giganteus* Greef et Deu.) are two perennial rhizomatous grasses (PRGs), considered as promising sources of lignocellulosic biomass for renewable energy production. Although the agronomic performance of these species has been addressed by several studies, the literature dedicated to the crop water use of giant reed and miscanthus is still limited. Our objective was thus to investigate giant reed and miscanthus water use by assessing crop evapotranspiration (ET_c), crop coefficients (K_c) and water use efficiency (WUE). The study was carried out in central Italy and specifically designed water-balance lysimeters were used to investigate the water use of these PRGs during the 2010 and 2011 growing seasons. Giant reed showed the highest cumulative evapotranspiration, with an average consumption of approximately 1100 mm, nearly 20% higher than miscanthus (900 mm). Crop evapotranspiration rates differed significantly between the species, particularly during the midseason (from June to September), when average daily ET_c was 7.4 and 6.2 mm in giant reed and miscanthus respectively. The K_c values determined in our study varied from 0.4 to 1.9 for giant reed and 0.3 to 1.6 for miscanthus. Finally, WUE was higher in miscanthus than in giant reed, with average values of 4.2 and 3.1 g L^{-1} respectively. Further studies concerning water use under nonoptimal water conditions should be carried out and an assessment of the response to water stress of both crops is necessary to integrate the findings from this study.

Keywords: crop water use, energy crops, evapotranspiration, lignocellulosic biomass, lysimeter, perennial rhizomatous grasses, water requirement, WUE

Received 19 August 2013 and accepted 8 January 2014

Introduction

Highly productive perennial grasses are considered as sources of lignocellulosic biomass, a renewable feedstock that contributes to reducing the use of fossil resources for the production of energy, chemicals and materials (Karp & Shield, 2008; Hulle *et al*., 2010).

Giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus × giganteus* Greef et Deu.) are two perennial rhizomatous grasses (PRGs) considered among the most promising crops for biomass production in southern Europe due to their high yield and low input requirements (Lewandowski *et al*., 2003; Angelini *et al*., 2009). Giant reed is a C3 grass that is wide spread in the riparian areas of the Mediterranean and found over a wide range of subtropical and warm-temperate areas of the world (Rossa *et al*., 1998; Lewandowski *et al*., 2003; Czako & Marton, 2010). Miscanthus is a C4 grass originating in east Asia which has been extensively studied in Europe since the early 1980s due to its high yielding capacity and cold tolerance (Beale *et al*., 1996; Anderson *et al*., 2011).

In the Mediterranean environment, both species present similar biomass accumulation trends and growth cycles, with a growing season starting in March–April and ending in October–November (Nassi o Di Nasso *et al*., 2011a). Studies carried out in this environment under rainfed conditions have confirmed the high long-term productivity of giant reed and miscanthus (38 and 28 t d.w. ha yr^{-1} respectively) in soils characterized by good nutrient and water availability (Angelini *et al*., 2009). However, crop productivity is not the only aspect to take into account when assessing the sustainability of lignocellulosic biomass production from dedicated crops. Water, nutrients and light are the three major resources needed for biomass production, and of these, water is often the most limited in the Mediterranean environment. In fact, precipitation events in the summer are scarce and the risk of drought is high. Moreover,

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there are concerns regarding the availability of water resources in the near future. In fact, questions have been raised about the implications of large-scale bioenergy production and their possible ecohydrological impact (Berndes, 2002; Powlson *et al.*, 2005; King *et al.*, 2013). In the case of giant reed and miscanthus, the use of water resources seems to be one of main aspects which differentiates the two species: giant reed can survive under very wet or dry conditions for a long period (Rossa *et al.*, 1998; Lewandowski *et al.*, 2003; Czako & Marton, 2010), while miscanthus does not tolerate prolonged drought and responds to water stress by senescing, losing leaf area and increasing root growth with respect to rhizome growth (Lewandowski *et al.*, 2000; Karp & Shield, 2008). In addition, giant reed is a C3 species, while miscanthus is a C4. It is known that C4 plant species tend to be more efficient in their use of water because they avoid photorespiration, while C3 species need more water than C4 species to produce the same amount of biomass (Sage & Monson, 1998).

Despite the relevance of the topic and the increasing interest in these two PRGs, only a few works have focused on investigating the water use of giant reed and miscanthus, and a direct comparison of both crops has not been undertaken. Previous studies have shown that the overall seasonal water demands of giant reed and miscanthus can be very high. For giant reed, Tzanakakis *et al.* (2009) reported cumulative evapotranspiration values between 1000 and 1500 mm yr^{-1} under nonlimiting water conditions, while Hickman *et al.* (2010) estimated values close to 1000 mm yr^{-1} in miscanthus, against ca. 600 mm yr^{-1} in maize. Evapotranspiration of PRGs is higher than annual crops (e.g., maize, sorghum), as PRGs grow over a longer season and build up a high above-ground biomass, a large leaf area and an extensive and deep root system (Finch & Riche, 2008; Nassi o Di Nasso *et al.*, 2011a). Some studies have also investigated the relation between water use and biomass yield of giant reed and miscanthus (Beale *et al.*, 1999; Christou *et al.*, 2003; Hickman *et al.*, 2010) but a comparison of the results is often difficult due to methodological differences.

An investigation of the crop water use of giant reed and miscanthus is thus essential to better understand the suitability and sustainability of these PRGs. Furthermore, quantitative information on crop evapotranspiration dynamics is fundamental for many agricultural and environmental applications, such as irrigation scheduling, implementation of crop growth models and hydrological studies (Rana *et al.*, 2001).

Our research objective was to compare the water use of giant reed and miscanthus under nonlimiting conditions. More specifically, the study was conducted using specifically designed water-balance lysimeters to determine: (i) the ten-day crop evapotranspiration rates (ET_{c}) and the seasonal crop evapotranspiration ($\sum ET_{c}$); (ii) the crop coefficient (K_c) in relation to crop growth stages; and (iii) the water use efficiency (WUE). The study was conducted in established stands of giant reed and miscanthus over two growing seasons in Pisa, central Italy.

Materials & methods

Lysimeter set-up and crop management

The research was conducted in 2010–2011 at the Enrico Avanzi Interdepartmental Centre for Agro-Environmental Research of the University of Pisa (CIRAA), located in the Pisa coastal plain, central Italy (43°N 10°E long; altitude 2 m a.s.l.).

Our lysimeter system was installed at the experimental site in spring 2009 and consisted of six water-balance lysimeters. Each lysimeter (Fig. 1) consisted of a PVC box 0.90 m deep, 1.2 m long and 1.2 m wide. Each box was first filled with a 5 cm high layer of expanded clay pebbles. On top of this, a non-woven fabric (geotextile) layer was laid and the box was then filled to the top with the original soil removed from the site (Table 1). A plastic pipe (0.2 m diameter, 2.2 m length) was installed vertically in the corner of each lysimeter. The pipe was placed touching the bottom of the box and the lowest end was drilled with small holes (5 mm diameter) to allow a water flow between the pipe and the bottom of the lysimeter. Inside each pipe, an automatic irrigation and drainage system was installed, consisting of two floating switches, a drainage pump, two mechanical pulse counters, an electronic command unit and two pipes, one for irrigation and one for drainage. The automatic system was designed to maintain an artificial 'water table' at 0.8 m depth throughout the growing season. When evapotranspiration led to a lowering of the water table level below a predetermined minimum level, a floating switch turned on the irrigation system. Similarly, when the water table exceeded a maximum level due to precipitation events, the excess water was drained by the drainage pump. In this way, soil moisture in the lysimeters was constantly maintained close to field capacity. Irrigation and drainage amounts were recorded twice daily by the mechanical pulse counters.

In April 2009, giant reed and miscanthus were transplanted into the lysimeters using micropropagated plants and rhizomes, respectively. The experimental layout adopted was a completely randomized design with three replications. Both species were grown in 0.5 × 1 m wide rows (2 plants m^{-2}). An area of 100 m^2 was planted around each lysimeter with the same crop to prevent 'clothesline' and 'oasis' effects (Allen *et al.*, 1998). This area was maintained in comparable water conditions to the crops in the lysimeters with a drip irrigation system.

In the establishment year, the crops were fertilized 15 days after planting at a rate of 150 kg N ha^{-1} (urea), 150 kg P_2O_5 ha^{-1} (triple superphosphate) and 150 kg K_2O ha^{-1} (potassium sulphate). Fertilization was repeated with the same doses each year in early March, before sprouting. Weed control was needed only in the establishment year and was performed manually.

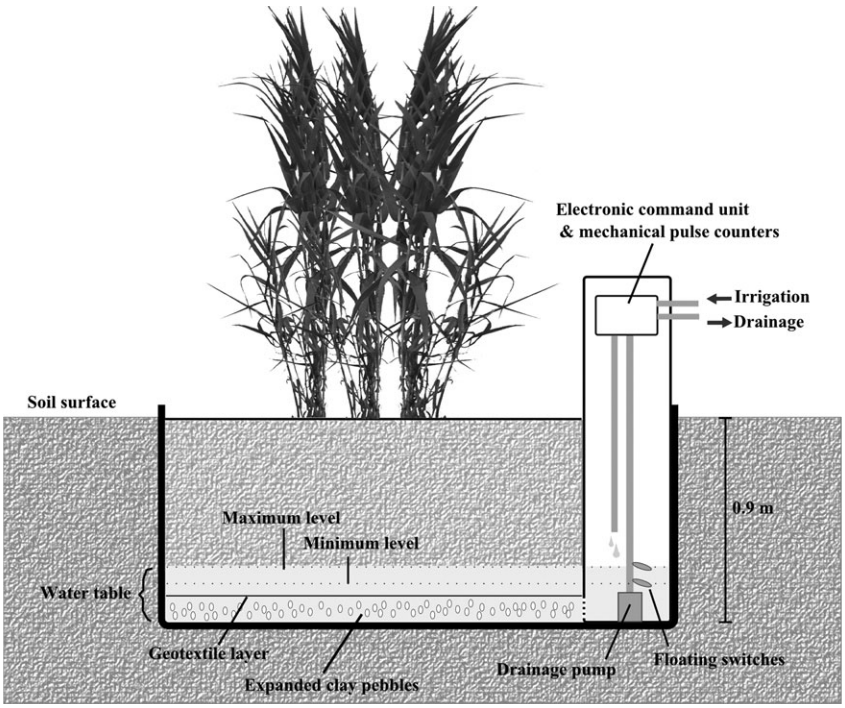

Fig. 1 The drainage lysimeters installed in 2009 at the experimental site (Pisa, Italy).

Table 1 Soil characteristics in the lysimeters at the experimental site (0–90 cm; March 2009)

Soil type (USDA soil classification)	Loamy sand
Clay (%)	8.0
Silt (%)	11.8
Sand (%)	80.2
pH	7.8
Field capacity (vol.%)	16.2
Wilting point (vol.%)	3.2
Available water content (vol.%)	13.0
Organic matter (%)	1.6

Crop development and biomass production

During the second and third growing seasons (2010 and 2011), crop development was monitored and four different growth stages were identified:

1. Initial: from crop sprouting to the beginning of stem elongation.
2. Crop development: stem elongation.
3. Midseason: from the end of stem elongation to the beginning of canopy senescence.
4. Late season: from canopy senescence to the end of water uptake.

The crops in the lysimeters and in the surrounding area were harvested yearly in autumn (November). Autumn harvest was preferred in this study, so that the biomass yield could be determined before a significant loss of biomass occurred. At harvest, the above-ground fresh biomass in the lysimeters was assessed. Subsequently, above-ground dry yield (AGDY) was determined by drying a subsample in a forced-air drier at 60 °C to constant weight.

Reference evapotranspiration

The reference evapotranspiration (ET$_0$) was calculated according to the FAO Penman-Monteith method (Allen *et al*., 1998). The Penman–Monteith equation was used to calculate the daily ET$_0$ (mm d^{-1}) using meteorological data recorded by an automatic weather station located near the experimental site (<500 m distance), as follows:

$$\mathrm{ET}_0 = \frac{0.408\Delta(R_\mathrm{n} - G) + \gamma \frac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34 u_2)} \quad (1)$$

where, R_n is the net radiation at the crop surface (MJ m^{-2} per day), G is the soil heat flux (MJ m^{-2} per day), T is the average air temperature (°C), u_2 is the wind speed at 2 m height (m s^{-1}), e_s is the saturation vapour pressure (kPa), e_a is the actual vapour pressure (kPa), Δ is the slope of the vapour pressure curve (kPa °C^{-1}), and γ is the psychrometric constant (kPa °C^{-1}).

Crop water use

In 2010 and 2011, crop evapotranspiration (ET$_c$) of giant reed and miscanthus was determined by calculating the hydrological balance of each lysimeter on a ten-day basis, from sprouting to the end of water demand (Jensen *et al*., 1990; Rana & Katerji, 2000), as follows:

$$\mathrm{ET_c} = I + R - D \pm \Delta S \tag{2}$$

where, I and D are the irrigation and drainage measured by the lysimeter system (mm) respectively, R is the precipitation recorded by the automatic weather station (mm), and ΔS is the variation in soil moisture content (mm). The latter was considered equal to zero over the ten-day interval, as the lysimeters were designed to maintain the soil moisture constant. The cumulative evapotranspiration for the entire growing season ($\sum ET_c$) was also calculated.

The crop coefficient (K_c) was calculated for each growth stage according to the single crop coefficient method (Jensen *et al*., 1990; Allen *et al*., 1998) from the ET_c calculated by the water balance method and ET_0 estimated by the Penman-Monteith method, as follows:

$$\mathrm{K_c} = \frac{\mathrm{ET_c}}{\mathrm{ET_o}} \tag{3}$$

Finally, the water use efficiency (WUE) of both crops was calculated as the ratio of the above-ground dry yield at harvest to the cumulative evapotranspiration:

$$\mathrm{WUE} = \frac{\mathrm{AGDY}}{\sum \mathrm{ET_c}} \tag{4}$$

Data analysis

For each growing season, data on crop growth and water use were subject to a one-way analysis of variance (ANOVA) to test the differences between the species, according to a completely randomized experimental design. The statistical analysis was performed using R software (R Core Team, 2012).

Results

Meteorological conditions

Monthly meteorological conditions of the two growing seasons (from March to October) are summarized in Table 2. Seasonal mean temperature was about 18 °C, while minimum and maximum values ranged between 4 and 31 °C. During the 2010 growing season, rainfall was about three times higher than in 2011 (1100 vs. 350 mm). This difference was mainly due to the abundant rainfall in 2010 in the summer and autumn periods. When rainfall is compared to the 20-year long-term average (380 mm, from March to October) it is possible to observe that 2010 was a particularly wet year, while 2011 was slightly drier. Although precipitation varied between the two seasons, no significant differences were registered in solar radiation. The only exception was in May, when 2010 values were 15% lower than 2011. In general, vapour pressure deficit (VPD) showed higher values in 2011 (+17%), with the exception of July. On average, seasonal ET_0 was similar in both years: 3.3 and 3.5 mm d^{-1} in 2010 and 2011 respectively. However, differences in monthly ET_0 were observed from May to October (±10%).

Table 2 Climatic conditions at the experimental site (Pisa, Italy) during the 2010 and 2011 growing seasons: average monthly maximum, minimum and mean air temperature (T_{max}, T_{min}, T_{mean}), total monthly rainfall (Rain), average monthly solar radiation (Rad), average monthly vapour pressure deficit (VPD), and average monthly reference evapotranspiration (ET_0)

	T_{max} (°C)	T_{min} (°C)	T_{mean} (°C)	Rain (mm)	Rad (MJ m^{-2} d^{-1})	VPD (kPa)	ET_0 (mm d^{-1})
2010							
March	14.6	4.7	9.6	36.6	9.9	0.45	1.9
April	19.4	6.3	12.8	77.2	16.5	0.66	2.8
May	21.5	10.1	15.8	176.6	16.6	0.68	3.1
June	26.1	14.0	20.1	68.0	21.4	0.91	4.2
July	31.5	17.1	24.3	103.4	23.0	1.34	5.1
August	28.8	16.2	22.5	100.4	19.7	1.07	4.0
September	27.5	12.0	19.7	54.8	14.4	1.14	3.1
October	20.6	9.7	15.1	230.9	9.0	0.65	1.9
Season average	23.8	11.2	17.5	–	16.3	0.9	3.3
Total rainfall (mm)				1111.2			
2011							
March	14.9	4.1	9.5	122.4	11.3	0.54	2.1
April	20.7	6.5	13.6	21.0	16.7	0.75	2.9
May	25.6	8.8	17.2	8.4	22.5	1.09	4.4
June	27.5	14.6	21.1	32.2	21.9	1.01	4.5
July	28.7	16.2	22.4	26.8	21.2	1.06	4.5
August	30.9	15.5	23.2	4.0	20.3	1.39	4.4
September	28.5	14.5	21.5	70.4	14.8	1.12	3.0
October	22.3	7.6	15.0	28.4	10.2	0.92	2.3
Season average	24.9	11.0	17.9	–	17.4	1.0	3.5
Total rainfall (mm)				346.8			

Plant growth and development

Slight differences were observed between the two species concerning cycle length, while there were substantial variations in AGDY. Giant reed showed a longer cycle than miscanthus (210 vs. 199 days). Giant reed sprouted on 29 March and 20 March in 2010 and 2011, respectively, about 10 days earlier than miscanthus (8 April 2010 and 31 March 2011). The initial-stage and the crop-development stage showed similar length in both crops, while the main differences were observed in the midseason stage (+20 days in giant reed) and in the late-season stage (+10 days in miscanthus) (Table 3). The midseason stage was the longest in both crops, and spanned from June to September. Miscanthus flowered at the beginning of the late season (6 September and 31 August in 2010 and 2011, respectively); at the same time canopy senescence increased rapidly, leading to almost all the leaves dying by the end of this stage. Giant reed on the other hand, did not flower and during the late season stage leaf loss was moderate.

In 2010, when crops were two years old, significant differences in AGDY were observed between the two species. The highest AGDY was observed in miscanthus (4300 vs. 3400 g d.w. m^{-2}) (Fig. 2). In 2011, no significant differences were observed between the species, and a mean AGDY value around 3000 g d.w. m^{-2} was recorded. In both years, giant reed showed a higher leaf mass ratio (i.e. ratio between leaf dry yield and AGDY) compared to miscanthus. Moreover, both species registered a higher leaf mass ratio in 2011 than in 2010 (in 2010: 22% vs. 4%; in 2011: 34% vs. 9% for giant reed and miscanthus respectively).

Crop water use

Results of the crop water balance highlighted a different water use of miscanthus and giant reed (Table 4). Cumulative ET_0 ($\sum ET_0$) showed similar values in 2010 and 2011, around 750 mm on average, while considerable differences in cumulative rainfall ($\sum R$) were observed between the two seasons (+480 mm in 2010). Consequently, cumulative drainage varied markedly between the years (around 470 and 75 mm in 2010 and 2011 respectively), with significant differences between the species occurring in 2010 (+60% in miscanthus respect to giant reed). In giant reed, cumulative irrigation water supply ($\sum I$) presented similar values in 2010 and 2011 (849 and 874 mm), while in miscanthus, it decreased from 903 to 672 mm determining significant differences between the two crops in 2011.

For both crops, the highest values of cumulative crop evapotranspiration ($\sum ET_{c.}$) were observed in 2010, +14% and +22% compared to 2011 in giant reed and miscanthus respectively. In general, giant reed showed higher values than miscanthus, although significant differences were observed only in 2011, when crops were 3 year old (1083 vs. 878 mm as mean values of 2010 and 2011 in giant reed and miscanthus respectively). On average, $\sum ET_{c.}$ was 30% and 15% higher than $\sum ET_0$ in giant reed and miscanthus respectively.

In general, miscanthus showed a higher water use efficiency (WUE) than giant reed (Table 4). However, statistical differences between the species were recorded only in 2010 (4.3 vs. 2.9 g L^{-1} in miscanthus and giant reed respectively), while in 2011, similar values were registered for both crops (about 3.5 g L^{-1}).

Figure 3 reports the reference evapotranspiration and crop evapotranspiration patterns for the 2010 and 2011 growing seasons. Seasonal ten-day $ET_{c.}$ dynamics were similar in both crops, although in general, giant reed showed higher $ET_{c.}$ than miscanthus, with the largest differences occurring from June to September. In 2010, from the initial to the crop-development stages (March–May) $ET_{c.}$ of giant reed and miscanthus showed the

Table 3 Growth stages (beginning date, duration) and total length of the 2010 and 2011 growing seasons

	Stage beginning date		Stage duration (days)		
Growth stages	Giant reed	Miscanthus	Giant reed	Miscanthus	Average length (days)
2010					
Initial	29 March	8 April	44	48	46
Crop development	12 May	7 May	25	22	24
Midseason	6 June	29 May	103	104	104
Late season	17 September	11 September	34	41	38
Total			206	215	210
2011					
Initial	20 March	31 March	43	42	43
Crop development	21 May	12 May	22	22	22
Midseason	12 June	3 June	86	88	87
Late season	6 September	31 August	45	51	48
Total			206	215	210

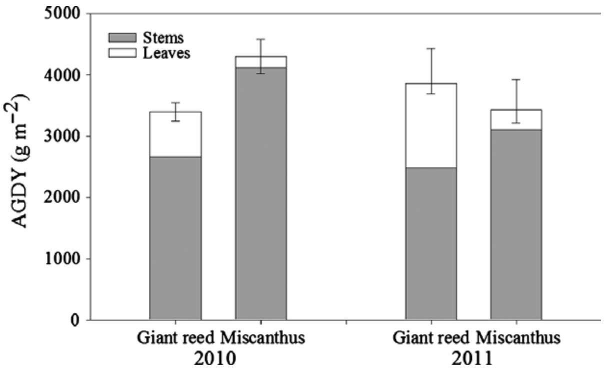

Fig. 2 Above-ground dry yield (AGDY) of giant reed and miscanthus at harvest in the 2010 and 2011 growing seasons (±SD).

same trend, increasing almost linearly (2.2 and 2.6 mm d^{-1} as mean values in the period for giant reed and miscanthus respectively). In the first half of the midseason (June–July), $ET_{c.}$ increased rapidly in both species until the end of July, when the highest $ET_{c.}$ was observed (12.4 and 10.7 mm d^{-1} in giant reed and miscanthus respectively). During this period, the growth rate of $ET_{c.}$ was about 0.10–0.15 mm d^{-1} in both crops. Subsequently, the second half of the midseason (July–September) was characterized by progressively decreasing $ET_{c.}$ values. During the midseason, $ET_{c.}$ of giant reed was higher on average than miscanthus (8.4 vs. 7.0 mm d^{-1} as mean midseason values). Finally, in the late season, $ET_{c.}$ decreased sharply until the end of the crop water demand. In 2011, ten-day $ET_{c.}$ showed similar trends as the previous year, although different $ET_{c.}$ values were recorded during the growing season. Higher values than 2010 were observed in the initial and crop-development stages for both crops (about 3 mm d^{-1} as mean value of the period), while lower maximum values were achieved at the end of July (10.9 and 7.9 mm d^{-1} in giant reed and miscanthus respectively). In addition, in the late-season stage (from September onwards), both crops exhibited lower values than 2010.

The 2010 and 2011 crop coefficients are reported for each growth stage in Table 5. In general, higher K_c values were observed in the 2010 growing season, except for the crop-development stage. In 2010, the K_c ranged from 0.67 to 1.93 and from 0.64 to 1.61 in giant reed and miscanthus respectively, while in 2011, values from 0.41 to 1.55 and from 0.31 to 1.20 were observed. Significant differences between the two species were recorded only in the midseason stage, when the K_c values of giant reed were around 20% higher than miscanthus.

Discussion

This study led to new insights on the water use under nonlimiting conditions of miscanthus and giant reed; two promising perennial rhizomatous grasses suitable for energy production. For both crops, the results highlighted a high water demand during the midseason (June–September), with K_c values greater than one. Considering the whole growing season, miscanthus was characterized by a lower $\sum ET_{c.}$ and a higher WUE than giant reed.

In the Mediterranean environment, several studies have been conducted on the agronomic management of these crops and on their suitability as energy feedstocks (Christou *et al.*, 2003; Angelini *et al.*, 2005; Cosentino *et al.*, 2007; Mantineo *et al.*, 2009; Anderson *et al.*, 2011; Nassi o Di Nasso *et al.*, 2011b). However, information on evapotranspiration dynamics and the water use of miscanthus and giant reed are still limited, and the few studies that have dealt with this specific issue differ greatly on the methodological approaches adopted, leading to some difficulties when comparing results (Beale *et al.*, 1999; Christou *et al.*, 2003; Finch & Riche, 2008; Tzanakakis *et al.*, 2009; Hickman *et al.*, 2010; McIsaac

Table 4 Water balance of giant reed and miscanthus in Pisa (Italy) in 2010 and 2011. Values (±SD) of cumulative reference evapotranspiration ($\sum ET_0$), cumulative rainfall ($\sum R$), cumulative irrigation ($\sum I$), cumulative drainage ($\sum D$), cumulative crop evapotranspiration ($\sum ET_{c.}$) and water use efficiency (WUE)

	$\sum ET_0$ (mm)	$\sum ET_{c.}$ (mm)	$\sum R$ (mm)	$\sum I$ (mm)	$\sum D$ (mm)	WUE (g L^{-1})
2010						
Giant reed	725	1161 ± 89	675	849 ± 101	359 ± 32	2.9 ± 0.1
Miscanthus	702	991 ± 88	666	903 ± 81	578 ± 44	4.3 ± 0.3
Significance		ns		ns	**	***
2011						
Giant reed	797	1004 ± 79	215	874 ± 48	84 ± 37	3.2 ± 0.3
Miscanthus	771	772 ± 30	167	672 ± 53	68 ± 29	3.7 ± 0.3
Significance		*		*	ns	ns

ns not significant; *, **, *** significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

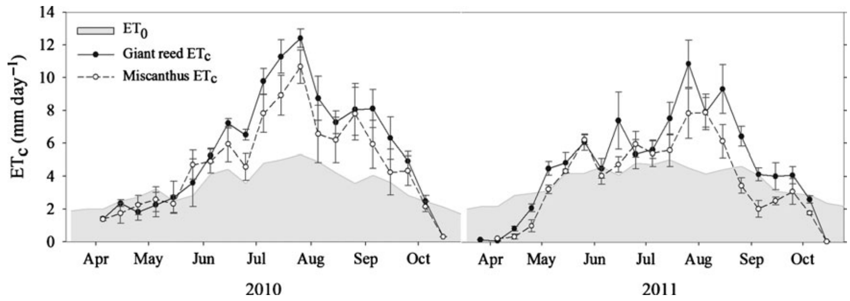

Fig. 3 Ten-day reference evapotranspiration (ET_0) and crop evapotranspiration (ET_c) of giant reed and miscanthus during the 2010 and 2011 seasons. Vertical bars represent standard deviations.

Table 5 Giant reed and miscanthus crop coefficient (K_c) (±SD) in 2010 and 2011 at the experimental site (Pisa, Italy)

	K_c (−)		
Year/Stage	Giant reed	Miscanthus	Significance†
2010			
Initial	0.67 ± 0.07	0.64 ± 0.09	ns
Crop development	0.91 ± 0.14	0.95 ± 0.14	ns
Midseason	1.93 ± 0.09	1.61 ± 0.14	*
Late season	1.38 ± 0.13	1.21 ± 0.18	ns
2011			
Initial	0.41 ± 0.06	0.31 ± 0.04	ns
Crop development	1.11 ± 0.14	1.14 ± 0.08	ns
Midseason	1.55 ± 0.14	1.20 ± 0.07	**
Late season	0.98 ± 0.12	0.68 ± 0.10	ns

†ns not significant; *, **, *** significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

et al., 2010; Watts & Moore, 2011). In our study, we used a water-balance lysimeter system, which enabled the incoming and outcoming crop water fluxes to be quantified. Similar methodological approaches have already been used to investigate water use of annual (Sepaskhah & Andam, 2001; Kang *et al.*, 2003) and perennial crops (Fronza & Folegatti, 2003; Skaggs *et al.*, 2006; Guidi *et al.*, 2008; Pistocchi *et al.*, 2009; Yarami *et al.*, 2011). However, this kind of approach had not yet been used for perennial energy grasses. In our experiment, a specifically designed lysimeter system, based on maintaining a constant level of soil humidity, was used to assess crop evapotranspiration through a simplified water balance. Thus, our lysimeter system was designed to determinate $ET_{c.}$ without measuring soil moisture variations over time, while quantifying the water lost by drainage and excluding water inputs due to capillary rise.

As reported by Nassi o Di Nasso *et al.* (2011a) for the same environment, above-ground dry yields over 30 t ha^{-1} yr^{-1} were observed in both species. In addition, the differences in AGDY we observed between 2010 and 2011 (second and third growing seasons) followed the trends already registered by Angelini *et al.* (2009) in a long-term study comparing the productivity of giant reed and miscanthus. Therefore, our results confirmed the importance of crop age in determining the yield level of giant reed and miscanthus.

In our study, both crops attained high cumulative crop evapotranspiration values. Giant reed showed the highest $\sum ET_{c.}$, which was on average nearly 20% higher than miscanthus. Compared to our findings, in the Mediterranean environment Christou *et al.* (2003) reported similar values of $\sum ET_{c.}$ in 2 and 3 year old irrigated giant reed stands (about 1000 mm). On the other hand, Tzanakakis *et al.* (2009) observed higher $\sum ET_{c.}$ values (+40%). Regarding miscanthus, no studies have been conducted in the Mediterranean environment, while in Midwestern USA Hickman *et al.* (2010) and McIsaac *et al.* (2010) estimated $\sum ET_{c.}$ values of 400 and 950 mm under rainfed conditions. The PRGs analysed in our study showed a much higher cumulative crop evapotranspiration than annual herbaceous crops suitable for energy uses such as maize and sorghum, which attain values between 450 and 650 mm yr^{-1} (Piccinni *et al.*, 2009; Hickman *et al.*, 2010). The higher water requirement of PRGs could be related to their longer growing season compared to annuals. In fact, in the Mediterranean environment the growth season of miscanthus and giant reed is about 210 days, as opposed to 150 days for summer annual crops (Piccinni *et al.*, 2009). In addition, during the midseason, the $ET_{c.}$ of giant reed and miscanthus was higher than the reference evapotranspiration ($K_c >> 1$) and lasted for about 80–100 days, while in maize and sorghum this period is 40–60 days (Allen *et al.*, 1998).

When comparing miscanthus and giant reed directly, the main differences in $ET_{c.}$ occurred during the midseason: average stage $ET_{c.}$ was 17% higher in giant reed. The higher $ET_{c.}$ of giant reed could be due to its longer midseason (+20 days) and its C3 photosynthetic pathway. Indeed, miscanthus, being a C4 crop, showed a more efficient use of water (WUE: 4.2vs. 3.1 g L^{-1}). For miscanthus, Hickman *et al.* (2010) reported a WUE of 1.9 g L^{-1} based on the winter above-ground dry yield. However, when referring to the autumn harvest, higher WUE values could be estimated (3.15 g L^{-1}). Taking this into account, the values reported by Hickman *et al.* (2010) are directly comparable with ours, showing a difference of about 20%. In addition, other studies reported in literature show miscanthus WUE ranging from 2.3 to 9.5 g L^{-1} (Beale *et al.*, 1999; Clifton-Brown & Lewandowski, 2000; Jorgensen & Schelde, 2001; Mantineo *et al.*, 2009). Such differences could be related both to the different methods used for measuring the water fluxes and the time at which AGDY is determined. Indeed, several studies have highlighted significant losses in miscanthus dry biomass when delaying harvesting from autumn to winter (from 30% to 50%) (Lewandowski *et al.*, 2000). On the other hand, overwinter biomass losses in giant reed have been observed to be very modest or absent (Angelini *et al.*, 2005), implying that sampling time is less problematic when calculating giant reed WUE. Regarding the latter, data on WUE are available from southern Europe trials: our results are in accordance with those reported by Christou *et al.* (2003) under irrigated conditions, while higher values (>4 g L^{-1}) were observed by Mantineo *et al.* (2009). Overall, the differences in WUE estimates suggest that the environmental conditions of the study site and the methodological approaches could play a crucial role in its evaluation.

In conclusion, our study showed that miscanthus was characterized by higher water use efficiency and lower cumulative crop evapotranspiration than giant reed. Both species showed higher water requirements during the midseason (June–September), which represented round 60–70% of the cumulative crop evapotranspiration of the growing season. In the same period, K_c reached its maximum, with values ranging from 1.5 to 1.9 and from 1.2 to 1.6 in giant reed and miscanthus respectively. Respect to annual crops, our results confirmed the higher water use efficiency of PRGs. However, further studies are necessary to investigate the behaviour of these crops in rainfed conditions. In fact, water use dynamics may change greatly when water becomes a limiting factor depending on genetic, physiological and morphological factors that drive crops' adaptation to drought. In addition, we believe there is a need to define standardized methods for characterizing crop water use of PRGs to reduce data variability and facilitate a comparison among the available data. Finally, our results provide valuable information for planning an efficient water management of these crops in the Mediterranean environment. The results could also be useful within the context of implementing crop growth models, for evaluating the impact of energy crops on hydrology and assessing the sustainability of energy crops at regional scale.

Acknowledgements

The authors thank Dr. Cristiano Tozzini and Fabio Taccini for their valuable field and technical support.

References

Allen R, Pereira L, Raes D, Smith M (1998) *Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements - Fao Irrigation and Drainage Paper 56*. FAO, Rome.

Anderson E, Arundale R, Maughan M, Oladeinde A, Wycislo A, Voigt T (2011) Growth and agronomy of *Miscanthus × giganteus* for biomass production. *Biofuels*, **2**, 167–183.

Angelini LG, Ceccarini 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.

Angelini LG, Ceccarini L, Nassi o Di Nasso N, Bonari E (2009) Comparison of *Arundo donax* L. and *Miscanthus × giganteus* in a long-term field experiment in Central Italy: Analysis of productive characteristics and energy balance. *Biomass and Bioenergy*, **33**, 635–643.

Beale CV, Bint DA, Long SP (1996) Leaf photosynthesis in the C4-grass *Miscanthus × giganteus*, growing in the cool temperate climate of southern England. *Journal of Experimental Botany*, **47**, 267–273.

Beale CV, Morison JI, Long SP (1999) Water use efficiency of C4 perennial grasses in a temperate climate. *Agricultural and Forest Meteorology*, **96**, 103–115.

Berndes G (2002) Bioenergy and water—the implications of large-scale bioenergy production for water use and supply. *Global Environmental Change*, **12**, 253–271.

Christou M, Mardikis M, Alexoupoulo E, Cosentino SL, Copani V, Sanzone E (2003) *Environmental Studies on Arundo Donax*. Proceedings of the 8th International Conference on Environmental Science and Technology, James & James (Science Publishers) Ltd., London, UK, 102–110.

Clifton-Brown JC, Lewandowski I (2000) Water use efficiency and biomass partitioning of three different Miscanthus genotypes with limited and unlimited water supply. *Annals of Botany*, **86**, 191–200.

Core Team R (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Cosentino SL, Patanè C, Sanzone E, Copani V, Foti S (2007) Effects of soil water content and nitrogen supply on the productivity of *Miscanthus × giganteus* Greef et Deu. in a Mediterranean environment. *Industrial Crops and Products*, **25**, 75–88.

Czako M, Marton L (2010) Subtropical and Tropical Reeds for Biomass (Chapter 16). In: *Energy Crops*, (eds Halford NG, Karp A), pp. 322–340. The Royal Society of Chemistry, London.

Finch JW, Riche AB (2008) Soil water deficits and evaporation rates associated with Miscanthus in England. *Aspects of Applied Biology*, **90**, 295–302.

Fronza D, Folegatti MV (2003) Water consumption of the estevia (Stevia rebaudiana (Bert.) Bertoni) crop estimated through microlysimeter. *Scientia Agricola*, **60**, 595–599.

Guidi W, Piccioni E, Bonari E (2008) Evapotranspiration and crop coefficient of poplar and willow short-rotation coppice used as vegetation filter. *Bioresource Technology*, **99**, 4832–4840.

Hickman GC, Vanloocke A, Dohleman FG, Bernacchi CJ (2010) A comparison of canopy evapotranspiration for maize and two perennial grasses identified as potential bioenergy crops. *GCB Bioenergy*, **2**, 157–168.

Hulle S, Roldán-Ruiz I, Bockstaele E, Muylle H (2010) Comparison of different low-input lignocellulosic crops as feedstock for bio-ethanol production. *Sustainable Use of Genetic Diversity in Forage and Turf Breeding*, pp. 365–368. Springer, Netherlands.

Jensen ME, Burman RD, Allen RG (1990) *Evapotranspiration and Irrigation Water Requirements*. ASCE, New York, NY.

Jorgensen U, Schelde K (2001) *Energy Crop Water and Nutrient Use Efficiency*. Report for IEA Bioenergy, Task 17.

Kang S, Gu B, Du T, Zhang J (2003) Crop coefficient and ratio of transpiration to evapotranspiration of winter wheat and maize in a semi-humid region. *Agricultural Water Management*, **59**, 239–254.

Karp A, Shield I (2008) Bioenergy from plants and the sustainable yield challenge. *New Phytologist*, **179**, 15–32.

King JS, Ceulemans R, Albaugh JM *et al*. (2013) The Challenge of Lignocellulosic Bioenergy in a Water-Limited World. *BioScience*, **63**, 102–117.

Lewandowski I, Clifton-Brown JC, Scurlock JMO, Huisman W (2000) Miscanthus: European experience with a novel energy crop. *Biomass and Bioenergy*, **19**, 209–227.

Lewandowski I, Scurlock JMO, Lindvall E, Christou M (2003) The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy*, **25**, 335–361.

Mantineo M, D'Agosta GM, Copani V, Patanè C, Cosentino SL (2009) Biomass yield and energy balance of three perennial crops for energy use in the semi-arid Mediterranean environment. *Field Crops Research*, **114**, 204–213.

McIsaac GF, David MB, Mitchell CA (2010) Miscanthus and switchgrass production in Central Illinois: impacts on hydrology and inorganic nitrogen leaching. *Journal of Environment Quality*, **39**, 1790.

Nassi o Di Nasso N, Roncucci N, Triana F, Tozzini C, Bonari E (2011a) Productivity of giant reed (Arundo donax L.) and miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) as energy crops: growth analysis. *Italian Journal of Agronomy*, **6**, e22.

Nassi o Di Nasso N, Roncucci N, Triana F, Tozzini C, Bonari E (2011b) Seasonal nutrient dynamics and biomass quality of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) as energy crops. *Italian Journal of Agronomy*, **6**, e24.

Piccinni G, Ko J, Marek T, Howell T (2009) Determination of growth-stage-specific crop coefficients (KC) of maize and sorghum. *Agricultural Water Management*, **96**, 1698–1704.

Pistocchi C, Guidi W, Piccioni E, Bonari E (2009) Water requirements of poplar and willow vegetation filters grown in lysimeter under Mediterranean conditions: Results of the second rotation. *Desalination*, **246**, 137–146.

Powlson DS, Riche AB, Shield I (2005) Biofuels and other approaches for decreasing fossil fuel emissions from agriculture. *Annals of Applied Biology*, **146**, 193–201.

Rana G, Katerji N (2000) Measurement and estimation of actual evapotranspiration in the field under Mediterranean climate: a review. *European Journal of Agronomy*, **13**, 125–153.

Rana G, Katerji N, Perniola M (2001) Evapotranspiration of sweet sorghum: a general model and multilocal validity in semiarid environmental conditions. *Water Resources Research*, **37**, 3237–3246.

Rossa B, Tüffers AV, Naidoo G, Von Willert DJ (1998) *Arundo donax* L. (Poaceae) - A C3 species with unusually high photosynthetic capacity. *Botanica Acta*, **111**, 216–221.

Sage RF, Monson RK (1998) *C4 Plant Biology*. Academic Press, San Diego, CA.

Sepaskhah AR, Andam M (2001) Crop coefficient of sesame in a semi-arid region of I.R Iran. *Agricultural Water Management*, **49**, 51–63.

Skaggs TH, Poss JA, Shouse PJ, Grieve CM (2006) Irrigating forage crops with saline waters 1. volumetric lysimeter studies. *Vadose Zone Journal*, **5**, 815–823.

Tzanakakis VA, Paranychianakis NV, Angelakis AN (2009) Nutrient removal and biomass production in land treatment systems receiving domestic effluent. *Ecological Engineering*, **35**, 1485–1492.

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

Yarami N, Kamgar-Haghighi AA, Sepaskhah AR, Zand-Parsa S (2011) Determination of the potential evapotranspiration and crop coefficient for saffron using a water-balance lysimeter. *Archives of Agronomy and Soil Science*, **57**, 727–740.