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Aboveground Yield and Biomass Quality of Giant Reed (*Arundo donax* L.) as Affected by Harvest Time and Frequency

Federico Dragoni · Nicoletta Nassi o Di Nasso · Cristiano Tozzini · Enrico Bonari · Giorgio Ragaglini

Abstract Giant reed (*Arundo donax* L.) is a perennial rhizomatous grass producing high biomass yields in temperate and warm climates under rainfed and reduced input conditions. Harvest time and frequency typically affect the productivity and suitability for energy conversion of energy crops. In order to evaluate the effect of different cutting managements on biomass yield and quality of giant reed, three single harvest (SH) and six double harvest (DH) systems were compared. Biomass yield, leaf mass ratio, dry matter (DM), and ash content were assessed for each harvest. Over the 2 years of study, giant reed demonstrated good productivity levels both when harvested once a year and twice a year (about 30 Mg ha^{-1}) without significant differences between the treatments. Regarding double-cut regimes, overall yields were significantly reduced by delaying the second cut from autumn to winter (32.9 vs 30.2 Mg ha^{-1}), and the percentage of the first cut with respect to the overall yield varied from 55 to 80 %. Biomass quality was also significantly affected by harvest time and frequency. The biomass obtained in double harvest systems showed higher average moisture levels (about 40 % DM) and ash concentrations ranging from 4.7 to 8.7 %. In contrast, single harvest systems led to a drier biomass (47–57 % DM) and reduced mineral contents (3.4–4.8 % ash). The feasibility of double-cut management should therefore be considered in terms of the specific giant reed-based supply chain, with particular regards to the storage and conversion technology adopted.

F. Dragoni (✉) · N. N. o Di Nasso · C. Tozzini · E. Bonari · G. Ragaglini
Institute of Life Sciences, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy
e-mail: f.dragoni@sssup.it

E. Bonari
CRIBE–Centro di Ricerche Interuniversitario Biomasse da Energia, Via Vecchia Livornese 748, 56122 Pisa, Italy

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Introduction

Perennial rhizomatous grasses are potential bioenergy crops for several reasons. Perennial grasses produce high dry matter yields, use soil nutrients more efficiently compared with annual crops, mitigate soil erosion, and provide carbon sequestration services, helping to preserve soil quality [1, 2]. Of these crops, giant reed (*Arundo donax* L.) is a promising grass for energy purposes, given its positive environmental impact and interesting yield potential under reduced input, rainfed conditions, and on marginal soils [3–7]. *A. donax* is acknowledged as an invasive species of riparian zones, and particularly in the USA, where it is listed as a noxious weed in some states [8, 9]. In flood zones, giant reed propagates widely by layering, when the shoots are bent to the ground, and by fragmentation, when the rhizome and stem pieces are dispersed by river currents. At opposite, its invasiveness potential is low when managed as a field crop, since it does not produce viable seeds and the expansion via rhizomes has been found to be slow [10, 11].

Very high biomass dry yields (over 35 Mg ha^{-1}) were achieved under Mediterranean conditions when water was not limited [3, 12]. A long-term field trial carried out in central Italy identified three yielding phases: a yield-increasing phase from the first to the third year (up to 55 Mg ha^{-1} year^{-1} d.m.), a steady-phase from the fourth to the eighth year (about 40 Mg ha^{-1} year^{-1} d.m.), and a yield-decreasing phase from the ninth year onwards (about 25 Mg ha^{-1} year^{-1} d.m.) [3]. The study of the growth dynamic of giant reed in the same environment highlighted that the aboveground biomass yield usually peaks in autumn and is almost steady in winter [13–15].

Biofuel supply chains should aim to combine productivity and good-quality biomass. Both aspects are largely influenced by cropping practices, including harvest management [7, 13]. Harvest time influences not only yield, but also the dry matter content, the ash concentration, and other biomass quality traits of candidate energy crops [16–20]. The conventional harvest management of giant reed and other perennial grasses relies on a single harvest per year in autumn or after a winter frost, when aboveground organs are senescent, in order to improve fuel quality by lowering the concentration of detrimental elements through leaf loss, nutrient leaching, and translocation to the rhizomes [7, 16–18]. However, giant reed typically shows higher mineral and moisture concentrations at harvest compared with other perennial energy crops [21, 22]. Moreover, natural drying and accessibility to the field can be seriously hampered in winter and autumn under typical Mediterranean conditions [13].

Different biomass conversion technologies have different requisites and constraints for feedstock quality [23–26]. For instance, when the crops are aimed at thermochemical processes, quality requirements are largely different from those of anaerobic digestion, in which high quality biomass facilitates conversion into methane at anaerobic conditions [27, 28]. Crop maturity has a negative effect on specific methane yields [29], while juvenile traits (e.g., high proportion of leaves, high moisture content) tend to be detrimental for thermochemical processes and beneficial for anaerobic digestion [24, 25]. Specific harvest strategies are thus conceivable when the crops are addressed to different biomass conversion processes. Conversely, harvest management could be considered as a means to direct the crops towards different supply chains. Multiple harvests per year have also been proposed for some perennial rhizomatous grasses, in order to increase their versatility of use [19, 30–32] and to favor biological conversion [24, 27]. Increased harvest flexibility could help to deal with fluctuations of weather patterns and bioenergy market [32]. Moreover, the number of annual harvest days can be varied by different cutting managements, which impact on capital costs for machinery and storage, and thus on the average delivery cost of biomass [33].

To date, multiple harvests on giant reed were addressed by a few studies. Crop regrowth after first cut was observed by Sharma et al. [34], whose study considered double-cut management on natural stands of giant reed in India. Further, insights on biomass yield and quality as affected by harvest management of cultivated giant reed were provided by Ragaglini et al. [35], who observed that cutting giant reed in summer and harvesting again in autumn could be favorable for anaerobic digestion, since it could increase methane yield by about 20 % when compared with a single harvest.

Given the growing interest in giant reed for bioenergy and the difficulty to achieve biomass quality standards for thermochemical conversion using this crop [21, 22], new research questions may arise, concerning the cutting regime and its effects on biomass yields and suitability for different supply chains. There is still a lack of experience in the double harvesting of giant reed under reduced input and rainfed conditions. In addition, although biomass yield and quality can vary according both to first and second harvest times, to the best of our knowledge, no studies have investigated the effects of different second cut times on biomass production from perennial grasses.

We thus assessed the biomass yield and quality of giant reed grown under Mediterranean conditions as affected by different harvest systems. This study aimed to (i) assess the crop stand structure and regrowth capacity according to the harvest time, (ii) compare the productivity of double harvest systems with single harvest systems, and (iii) evaluate the biomass quality as affected by different harvest times and frequencies.

Materials and Methods

Site Description and Crop Management

This study was conducted in 2011 and 2012 at the Interdepartmental Centre of Agroenvironmental Research (CIRAA), San Piero a Grado, Pisa, Italy (43° 40′ 49.21″ North, 10° 20′ 47.15″ East; 1 m above mean sea level and 0 % slope). Giant reed was established in March 2006 on a typical xerofluvent soil, representative of the lower Arno River plain and characterized by a shallow water table. Soil samples were collected before planting and were analyzed for texture, pH, organic matter, total nitrogen, available phosphorus, and exchangeable potassium (Table 1). Tillage was conducted in the autumn of 2006 and consisted of medium-depth plowing (30–40 cm). Soil preparation was conducted in the spring, immediately before planting, by one pass with a double-disk harrow and one pass with a field cultivator. Establishment was performed using rhizomes with a couple of buds weighing about 500 g each, which were planted at a depth of 10–20 cm.

Giant reed was transplanted in 1-m-wide rows with a density of 20,000 rhizomes per hectare. In the establishment year,

Table 1 Soil characteristics (soil horizon 0–30 cm)

Sand	(%)	45.3
Silt	(%)	43.3
Clay	(%)	11.4
pH		7.9
Organic carbon (Walkley-Black)	(g kg^{-1})	13.0
Total nitrogen (Kjeldahl)	(g kg^{-1})	1.2
Assimilable phosphorus (Olsen)	(mg kg^{-1})	11
Exchangeable potassium (Dirks and Scheffer)	(mg kg^{-1})	131

fertilizers were distributed at a rate of 100 kg P_2O_5 ha^{-1} (triple super phosphate), 100 kg K_2O ha^{-1} (potassium sulfate), and 100 kg N ha^{-1} (urea). The nitrogen fertilizer was applied 50 % pre-plant and 50 % side dressing when plants were 0.30–0.40 m tall. In the following years, 100 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1} were applied in winter (around January), while 100 kg N ha^{-1} were applied entirely in late March, at the beginning of the growing season.

Cutting Regime and Measurements

From 2006 to 2010, giant reed was managed under a single harvest system, cutting on a single date in January. In the years 2011 and 2012, different harvest treatments were evaluated, considering double harvest and single harvest systems (Table 2). The crop was harvested in June, July, August, September, October, and January, from canopy closure to late senescence. Crop regrowth was observed in plots harvested in June, July, and August, thus leading to perform second cuts. These plots were split in two subplots and two different second cuts were carried out in autumn and in winter. Therefore, double harvest systems were made up by first cuts (FC) taking place in June, July, and August (DH1, DH2, DH3) and second cuts (SC) made in October and in January (DH-A, DH-W). The overall dry biomass yield for each double harvest system was obtained by summing the dry yields of first and second cuts, then compared with single harvests carried out in September, October, and January (SH-S, SH-A, and SH-W, respectively).

The plots were arranged according to a completely randomized design with three replications and were spaced by 2-m-wide strips, managed in order to avoid shadowing on harvested areas, thus having uniform conditions after cut. At harvest times, biomass fresh weight was determined on a sample of 2 m^2 in each plot, then the crop in the whole plot area (12 m×3 m) was cut 5 cm above the ground level. Giant reed biomass from the outer rows was never included in the harvested sample area. Biometric data, such as node number per shoot, shoot number per unit area, and plant height, were also collected within each sample. Plant samples were partitioned into stems and leaves, in order to determine the proportion of the different plant parts on the aboveground biomass. Dry matter (DM) content was obtained by oven drying at 60 °C until constant weight. Whole plant sub-samples were taken for ash determination, chopped using an electric powered shredder (AL-KO H1600), dried, and then milled (Retsch SM1) to a particle size <297 μm. Ash content was determined as loss on ignition after incineration of a 0.5-g sample in a muffle furnace at 550 °C according to the standard method EN 14775:2009.

Statistical Analysis

Biomass yields of the harvest systems were compared by mixed model analysis of variance, implemented in the R statistical environment (version 3.0.1) using the lmer function of the lme4 package [36]. Harvest system was considered as fixed effect, while year and replication were considered as random. *p* values for the fixed effect were estimated by the pamer.fnc function of the LMERConvenienceFunction package [37]. In addition, the role of cutting dates in determining the overall yield of double harvest systems was evaluated by considering first and second cuts as fixed effects, and year and replication as random effects. Differences in leaf mass ratio, DM concentration, and ash content were assessed by one-way analysis of variance for each year separately. Significance was determined at the $p<0.05$ level (LSD test). Linear models were applied in order to calculate correlations and identify regressions between (1) node number per shoot and plant height during the vegetative season, (2) leaf mass ratio and dry matter content, and (3) leaf mass ratio and ash content. Next, the significance of difference between first and second cuts was assessed by unpaired two-tailed Student's *t* test considering the slopes of the models.

Table 2 Harvest dates for the considered single harvest (SH) and double harvest (DH) systems

Harvest systems	Name	2011			2012		
		FC	SC-A	SC-W	FC	SC-A	SC-W
Double harvest (DH)	DH1-A	21 Jun	28 Oct	–	25 Jun	22 Oct	–
	DH2-A	15 Jul	28 Oct	–	12 Jul	22 Oct	–
	DH3-A	2 Aug	28 Oct	–	6 Aug	22 Oct	–
	DH1-W	21 Jun	–	16 Jan	25 Jun	–	20 Jan
	DH2-W	15 Jul	–	16 Jan	12 Jul	–	20 Jan
	DH3-W	2 Aug	–	16 Jan	6 Aug	–	20 Jan
Single harvest (SH)	SH-S	15 Sep	–	–	17 Sep	–	–
	SH-A	28 Oct	–	–	22 Oct	–	–
	SH-W	16 Jan	–	–	20 Jan	–	–

FC first cut, *SC* second cut; *-S* summer, *-A* autumn, *-W* winter

Results

Climate Conditions

Climate data of the considered period and monthly long-term average rainfall (1990–2010) are reported in Fig. 1. The mean temperatures during the growing seasons of 2011 and 2012 (April–October) were comparable to the 20-year average (18.5 °C), as well as the annual mean temperature (15 °C). The only notable exception was the autumn of 2012 which was about 1 °C warmer than the long-term average. The seasonal distribution of precipitation was markedly different in the considered years and atypical rainfall patterns were recorded in the two years of the experiment compared with the long-term meteorological data. Total annual rainfall was about 30 % lower than the long-term data in 2011 (626 vs 924 mm) and in line with the long-term in 2012 (935 mm). However, in 2012, rainfall events were mainly concentrated in the late months of the year, which accounted for more than 50 % of the annual precipitation. In both years, around 25 % of the annual precipitation occurred in the spring. In 2011, the summer period was about 30 % drier than the long-term (63 vs 94 mm), while in 2012 the summer was even drier (46 mm). In fact, the increase in accumulated precipitation from June to September was particularly modest in 2012 (about 50 mm). In 2011 and 2012, the autumn was 67 % drier (132 mm) and 12 % wetter (442 mm) than the long-term, respectively.

Biometry and Productivity

In both years, the shoot number per square meter did not significantly vary throughout the season (i.e., between SH systems and the first cuts of DH systems) (48 shoots m^{-2}), while a significant reduction was observed comparing first cuts with the second cuts of DH systems (49 vs 33 shoots m^{-2}) ($p<0.001$). No significant variation in shoot density was found between the two years, neither in SH, nor in DH. In contrast, plant height varied considerably between the two years and between the different harvest times (Fig. 2). Across the treatments, in 2012 plant height was reduced on average by about 25 % ($p<0.001$). Moreover, the shoots were significantly shorter when giant reed was harvested twice a year compared with single harvests (161 vs 254 cm) ($p<0.001$).

Analysis of variance on biomass yield indicated that single harvest systems performed similarly to double harvest systems over the 2 years of study, since no significant difference was shown (Table 3). Across the harvest treatments, giant reed yielded on average 36.5 Mg ha^{-1} in 2011 and 27.3 Mg ha^{-1} in 2012. Thus, biomass yields were substantially lower in the second year compared with the first year, both with double-cut management (−22 %) and conventional single-cut management (−29 %) (Fig. 3). Nevertheless, significant differences were found among double-cut managements, whose yields varied according to second cut date ($p<0.05$); there was no interaction between the first and second cut dates. In 2011, DH-A and DH-W systems averaged 37.2 and 34.0 Mg ha^{-1}, respectively, while the mean yield in SH systems was 38.3 Mg ha^{-1}. Analogously, biomass yield in 2012 was slightly higher in DH-A (28.6 Mg ha^{-1}) than in DH-W systems (26.3 Mg ha^{-1}), while biomass production in single-cut managements was intermediate (26.9 Mg ha^{-1}). Regarding double harvest systems, in 2011 the yields at first cut (FC) did not largely vary (23.5 Mg ha^{-1}), while in 2012 they increased from 14 Mg ha^{-1} in June to 24 Mg ha^{-1} in August. When the second cut was performed in autumn (SC-A), giant reed yield was lower by about 40 % in DH3 than in DH1 (Table 4). In addition, second cuts performed in winter (SC-W) showed an average decrease in biomass yield of about 3 Mg ha^{-1} with respect to SC-A (−35 %). In both years, the first cuts yielded a larger proportion of total harvested biomass, especially when

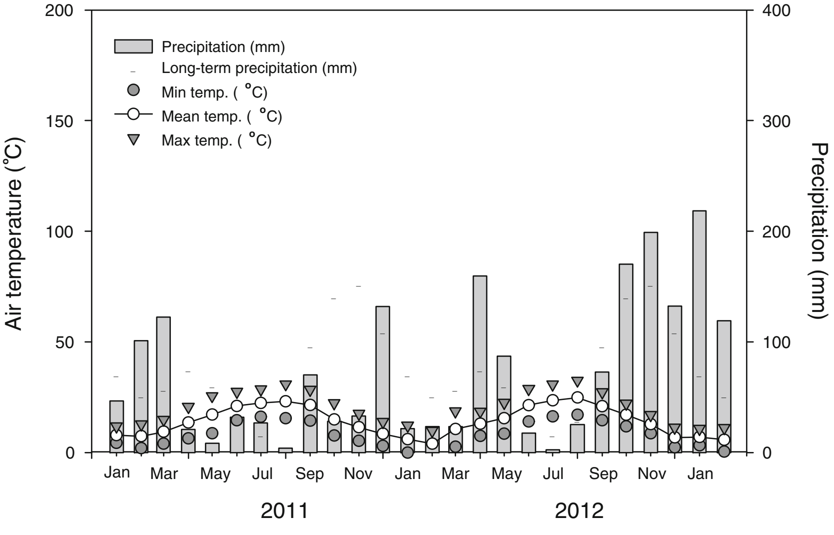


Fig. 1 Monthly and long-term precipitation, and minimum, maximum, and mean air temperature in San Piero a Grado (Pisa, Italy) for 2011 and 2012. The data were collected daily from a monitoring station located close to the field trial (3 m.a.s.l., 43° 40′ N, 10° 20′ E). The *chart* is presented as a Bagnouls and Gaussen diagram [38] in order to identify dry months (when precipitation is equal to or less than twice the monthly mean air temperature value, $p \leq 2$ T)

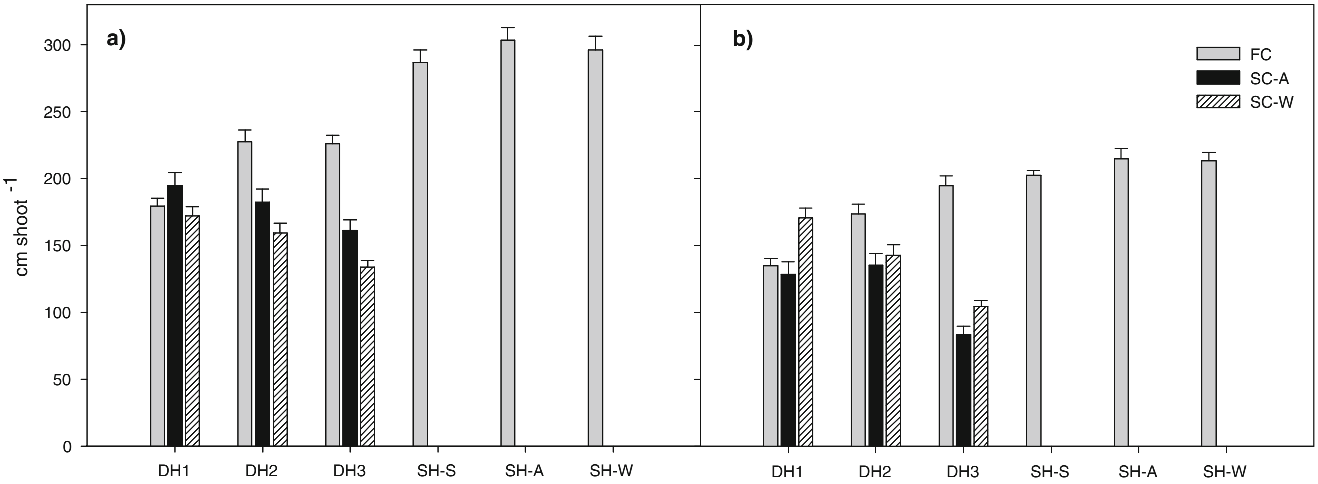


Fig. 2 Plant height of giant reed at the last ligulate leaf, as affected by different harvest systems during the years 2011 (**a**) and 2012 (**b**). *FC* first cut, *SC-A* second cut-autumn, *SC-W* second cut-winter. *Vertical bars* are the mean standard errors

first harvest was delayed. On average, the ratio between FC and overall DH yields increased from DH1-A to DH3-A (55–73 %), and a similar trend was observed from DH1-W to DH3-W (59–80 %).

Leaf loss along the season was shown by the progressive decrease in the dry leaf mass ratio from June to January (Table 5). It is worth nothing that no significant differences in leafiness were observed among FC and SC within double harvest systems, except in 2012, when particularly low leaf mass ratios were shown in SC-W. Likewise, in 2012 there was a noticeable decrease in leaf mass ratio in SH-W compared with SH-A.

Giant reed managed under DH systems showed a positive correlation between the number of nodes per shoot and the shoot height which was described by two statistically different linear regressions in FC and SC ($p<0.01$) (Fig. 4). Plants regrown after cutting (SC) were characterized by shorter internodes compared with plants of a similar height at first cut.

Table 3 Mixed model analysis of variance for biomass dry yield

Source of variation	Biomass yield			
	df	*F*	*P*	
Harvest system	8	0.832	0.580	ns[a]
	df	*F*	*P*	
FC	2	0.477	0.626	ns
SC	1	4.653	0.039	*
FC×SC	2	0.005	0.995	ns

The effect of the harvest system is reported in the upper part of the table, while the effect of first cut (FC), second cut (SC), and their interaction in double harvest system is reported in the lower part

*$p<0.05$; **$p<0.01$; ***$p<0.001$

[a] Not significant

Dry Matter and Ash Content

Dry matter (DM) and ash content were significantly affected by cutting time and frequency in both the years considered (Table 5). In general, DM content significantly increased over the season from June (28 %) to January (55 %). Greater DM contents were shown throughout the year 2011 compared with 2012 (46.6 vs 40.6 %) ($p<0.01$) and single-cut management led to the highest DM percentages (52 vs 41 %) ($p<0.001$). By cutting in June (DH1), the DM content at first harvest was 31.0 and 25.8 % in 2011 and 2012, respectively, while it was significantly higher at second cut. The same happened in both years by cutting in July and August (DH2 and DH3), although these differences were comprised in a narrower range and were not always significant. Thus, in the DH systems, the difference in DM content of the first and second cuts was progressively reduced by delaying the first harvest. Across the two years, the DM content of the second cuts also increased from autumn (40.1 %) to winter (48.8 %). In general, SC obtained in DH1 systems exhibited significantly higher dry matter levels compared with DH2 and DH3.

There was a steep decrease in ash content during both the seasons, from a high of 7.4–8.7 % in plants harvested in June (FC of DH1) to a minimum of 3.8–3.4 % in autumn/winter (SH-A and SH-W) (Table 5). Interestingly, in 2011 the ash content in SH-W was significantly higher than in SH-A and SH-S ($p<0.001$). Across the years, biomass from single harvest systems showed a markedly lower ash content than in double harvest systems (4.2 vs 6.5 %), while in second cuts the ash level was notably higher in autumn than in winter (7.1 vs 5.8 %) ($p<0.001$). No significant reduction in ash content was found from SC-A to SC-W in 2011 (6.6 vs 6.4 %), while there was a notable decrease in 2012 (7.7 vs 5.2 %) ($p<0.001$). In addition, in 2012, SC-A was significantly lower in minerals in DH1 than in DH2 and DH3, but such differences were not

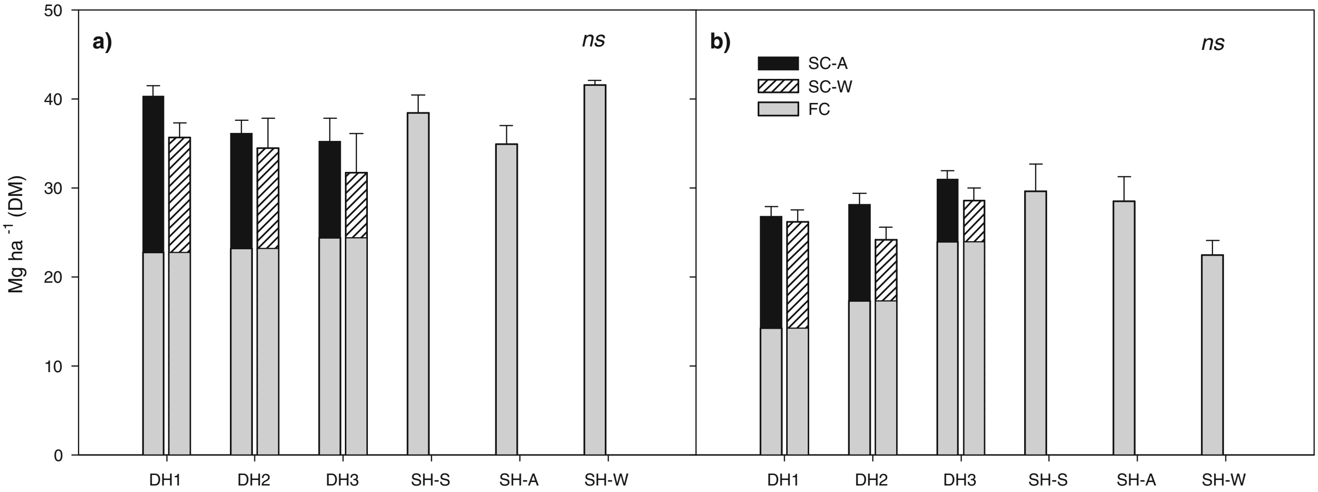


Fig. 3 Biomass dry yield of giant reed for the harvest systems in 2011 (**a**) and 2012 (**b**). *FC* first cut, *SC-A* second cut-autumn, *SC-W* second cut-winter. *Vertical bars* are the mean standard errors

evidenced in 2011. A similar trend was observed in SC-W in both years, since second cuts showed higher ash contents in DH3 compared with DH1 and DH2. First cuts (FC) were significantly richer in minerals compared with second cuts in DH1 systems only, while the highest mineral content in DH2 and DH3 was found in second cuts, and particularly in SC-A.

As shown in Fig. 5, variations in biomass quality can be explained by correlations among leaf mass ratio, ash, and DM content. We found that the higher the leaf mass ratio, the lower the DM and the higher the ash content. Different linear regressions were also identified for FC and SC compared with ash and DM content ($p<0.001$), showing that changes in biomass leafiness led to greater variations in first cuts than in second cut quality.

Table 4 Days after sprouting (DAS) and growing degree days (GDD) in the growing seasons 2011 and 2012 from sprouting to first cut (FC) and from FC to the autumn (SC-A) and winter (SC-W) second cuts, respectively

	2011			2012		
	FC	SC-A	SC-W	FC	SC-A	SC-W
DAS (day)						
DH1	93	128	208	90	119	209
DH2	117	104	184	107	102	192
DH3	134	87	167	132	77	167
SH-S	178			174		
SH-A	221			209		
SH-W	252			299		
GDD (°C)						
DH1	562	1378	1492	543	1469	1603
DH2	884	1056	1170	783	1229	1363
DH3	1070	870	984	1131	881	1015
SH-S	1700			1685		
SH-A	1940			2012		
SH-W	2054			2146		

GDD were calculated from sprouting according to the NOAA method, considering a base temperature of 10 °C and a cut-off temperature of 30 °C [3]. Dates of sprouting were 20 March and 27 March in 2011 and 2012, respectively

Discussion

Our study suggests that stable biomass yields can be obtained from mature stands of giant reed at different harvesting frequencies. Overall, yearly yields were not significantly affected by the different harvest treatments over the 2 years of study, since the double harvest and single harvest strategies led to similar productivity levels. In double harvest systems, second cuts compensated for the reduced yield achieved at first cut, while in single-cut management there was no substantial yield reduction from late summer to winter harvest.

On average, total annual dry yields were 40 and 25 Mg ha^{-1} in 2011 and 2012, respectively. In a previous study carried out in the same environment by Angelini et al. [3], giant reed reached crop maturity 4 years after planting, then biomass dry yields were found to be relatively steady for 4–5 years (38 Mg ha^{-1}). The present study was carried out on mature crops (6–7 years old): in 2011 yields were in line with Angelini et al. [3], while in 2012 they were lower. Despite generally being considered as resistant to water stress [6, 7], yield reductions due to summer droughts have been observed in Mediterranean environments by Nassi o Di Nasso et al. (2013) [5]. An intense summer drought occurred both in 2011 and 2012, but water scarcity was far more severe in the second year, despite an abundant rainfall recorded in spring. Thus, this may have caused a decrease in biomass yield with respect to the previous year, irrespectively of the harvest treatment, as evidenced by the reduced crop height. In fact, our

Table 5 Leaf mass ratio, DM concentration, and ash content reported by cut and year for the single harvest (SH) and double harvest (DH) systems

	2011						2012					
	FC		SC-A		SC-W		FC		SC-A		SC-W	
Leaf mass (% *w*/*w*)[a]												
DH1	33.9	aX	29.9	aX	23.9	aX	36.6	aX	37.7	aX	8.6	aY
DH2	28.0	abcX	29.2	aX	27.5	aX	32.3	aX	40.4	aX	13.4	aY
DH3	32.2	abX	28.6	aX	33.3	aX	24.6	bY	48.1	aX	17.8	aY
SH-S	25.2	bc					26.0	b				
SH-A	22.2	c					25.5	b				
SH-W	23.1	c					6.5	c				
DM (% *w*/*w*)												
DH1	31.0	dZ	48.3	aY	54.7	aX	25.8	eZ	37.6	aY	50.5	aX
DH2	37.9	cZ	45.3	abY	52.5	aX	32.6	dY	35.8	aY	46.0	aX
DH3	38.1	cY	42.6	bX	47.3	bX	41.5	cX	30.9	bY	42.0	aX
SH-S	50.9	b					47.7	b				
SH-A	57.3	a					46.6	b				
SH-W	55.9	a					54.7	a				
ash (% *w*/*w*)[a]												
DH1	7.4	aX	6.5	aY	6.1	bY	8.7	aX	7.0	bY	4.7	cZ
DH2	5.6	bY	6.3	aX	5.9	bY	6.9	bY	8.3	aX	5.1	bZ
DH3	5.7	bY	6.8	aX	7.2	aX	4.9	cZ	7.9	aX	5.7	aY
SH-S	4.0	d					4.8	c				
SH-A	3.8	d					4.8	c				
SH-W	4.4	c					3.4	d				

Upper and lower case letters are for comparison within the same row and the same column, respectively. All the comparisons were made within the single year. Values with the same letter on a row or a column are not significantly different at $p=0.05$

FC first cut, *SC-A* second cut-autumn, *SC-W* second cut-winter

[a] On a dry basis

results confirm those obtained in southern Italy by Mantineo et al. [12] on mature crops cultivated with an equivalent nitrogen fertilization level and low water availability. In addition, generally higher ash concentrations were observed

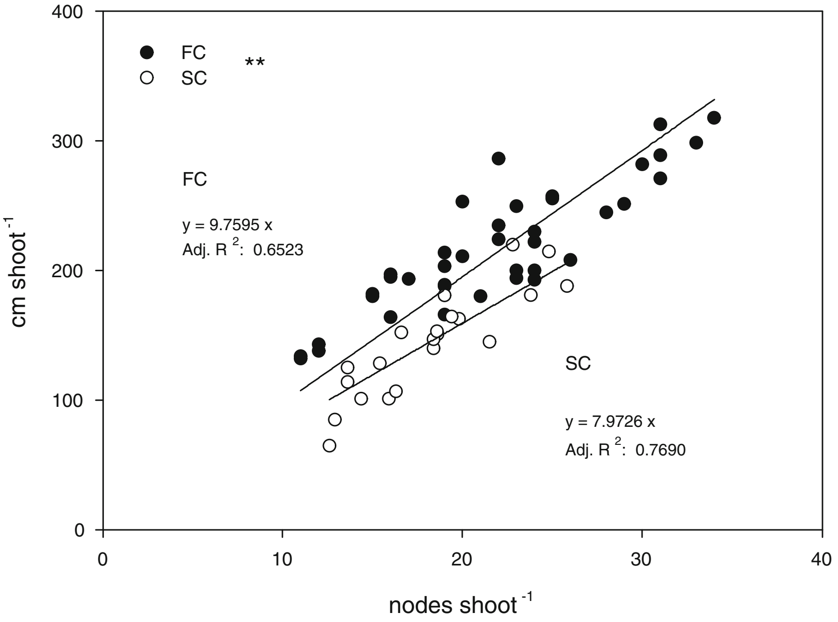
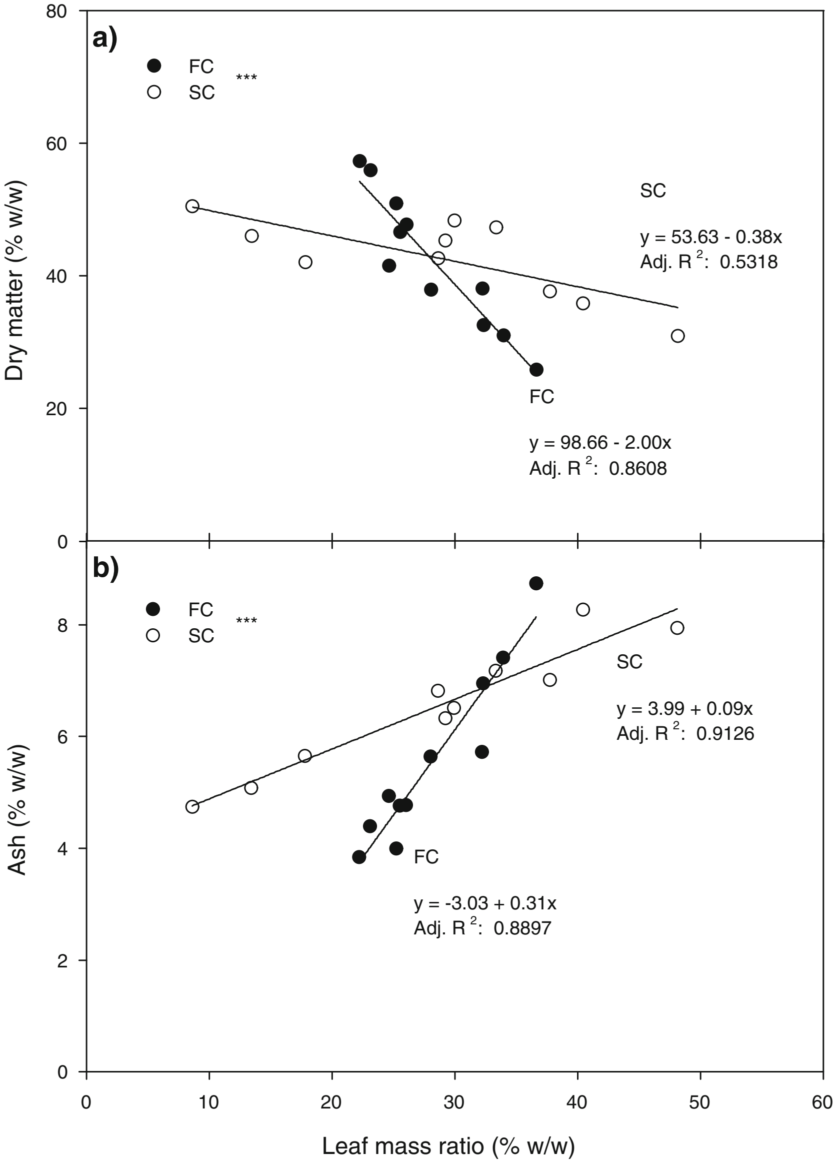


Fig. 4 Linear regressions of the correlations between node numbers per shoot and shoot height in FC (*black dots*) and SC (*white dots*) during the vegetative season. Differences between the slopes of the regression models were statistically significant (**$p<0.01$)

Fig. 5 Linear regressions of the correlations between leaf mass ratio and DM content (**a**) and leaf mass ratio and ash content (**b**) in FC (*black dots*) and SC (*white dots*). Ash content and leaf mass ratio were calculated on a dry matter basis. Differences between the slopes of the regression models were statistically significant (****p*<0.001)



during the second year, which may have been due to mineral accumulation occurring in order to relieve drought stress and favor water uptake [39].

No variations in shoot number per square meter were observed between the two years of the experiment, either in FC or in SC. Therefore, the yield component that explains the yield variations to the greatest extent is shoot height, which was generally reduced in 2012. In addition, stability in shoot density of double harvest systems between the two years suggests that in the short-term, the resprouting capability of giant reed was not affected by increased harvest frequency. Similar results have been found for lowland and upland cultivars of switchgrass (*Panicum virgatum* L.) grown under Mediterranean conditions, in which the shoot number per unit area did not change appreciably as a result of double-cut management over the first 2 years [32].

However, cutting during the vegetative season had a large impact on giant reed stand structure. Plants at second cut showed increased leafiness and shorter internodes with respect to plants harvested once. The newly formed leaf mass largely contributed to yield recovery after the early cut, and thus to the overall steadiness of the yield between double-cut and single-cut options.

A noticeable change in shoot density was also observed between FC and SC in both years. At early stages, rhizomatous species rely on belowground carbon sinks to support their growth, then the biomass increase is sustained by autotrophic activity of the newly formed shoots [40–42]. Giant reed is no exception, since the rhizome mass was found to decrease for some months from sprouting and to increase thereafter [5]. Therefore, repeated cutting during the season may have led to the partial depletion of rhizome carbohydrates, thus possibly affecting the giant reed's capacity to form new shoots, as hypothesized for the recurrent removal of aerial parts in this and other species [41, 42].

Interestingly, Sharma et al. [34] observed no shoot density changes in the giant reed at second cut compared with the same crop harvested once. They also noticed that the total aboveground yearly production was increased by cutting twice a year, while the belowground mass was negatively affected. However, their study was on natural stands of giant reed grown in very different climate conditions from those in our study.

Other studies on perennial crops have highlighted the contrasting effects of rainfall in limiting aboveground biomass yield under a two-cut management. For instance, while Fike et al. and Monti et al. [31, 32] observed that the rainfall course did not greatly affect the switchgrass response to harvest frequency, Guretzky et al. [30] referred to double-cut management in this species as a feasible option when rainfall is sufficient to sustain crop regrowth. Therefore, in our study, reduced water availability in the summer may have limited crop regrowth and modified the crop stand structure at the second harvest. However, a more definitive assessment of stability in biomass yield and shoot density of double harvest systems would require further investigation over longer periods.

We found a moderate biomass increase from SH-A to SH-W in 2011, while in 2012 there was a modest yield reduction in the same time interval. Interestingly, the slight yield increase recorded in 2011 was accompanied by an increase in ash content. This controversial behavior highlights the influence of the climatic conditions, in particular the minimum air temperature, on giant reed growth. Giant reed tends to conserve vital stems and leaves which may enable the plant to continue growing during the winter period [21, 22, 34]. Although the older leaves fall and the dry yield accumulation progresses slowly, the crop often does not undergo full senescence [13]. A proportion of the plant can remain green, especially under mild winter conditions that frequently occur in the Mediterranean.

Reduced senescence is consistent with the yield stability of giant reed throughout autumn and winter, which has also been reported in other studies [14, 15]. These "stay-green" traits can actively help sustain crop yields by extending the growing season [26]. Thus, the tendency to avoid senescence may support giant reed productivity in double harvest systems. In contrast, other perennial rhizomatous crops, such as miscanthus hybrids (*Miscanthus* x *giganteus*) and switchgrass, generally stop growing in the autumn and senesce, in relation to their cycle length, according to genotype and environmental factors. As a consequence, different grasses in different conditions are prone to different winter yield reduction (up to 40 %) [16–18, 22]. The lack of senescence may be linked to reduced leaf loss in giant reed, which is delayed and less pronounced compared with miscanthus hybrids in Mediterranean conditions [3, 14]. However, the marked decrease in leaf mass ratio observed between SC-A and SC-W in 2012 may have been triggered by heavier rainfall than in 2011, which also decreased the ash content.

Leaf mass ratio, DM, and ash concentrations varied in relation to the maturity level of the crop. The considered harvest times, leading to different DM contents, may influence giant reed-based supply chains in several ways, such as storage, logistics, and conversion. Increased harvest frequency caused the feedstock to have a higher moisture content than single harvest, as observed for other species [19, 32]. According to Smith and Slater [21], DM content should not be lower than 75 % when biomass is delivered to thermochemical conversion facilities. In our study, the moisture content exceeded 25 % at all the harvest times, with no exception for SH-A and SH-W, in line with the results obtained by Smith and Slater [21].

Comparing giant reed with switchgrass, Kering et al. [20] observed that overwintering caused a considerable loss of moisture in leaves of both crops, while drying out in giant reed stems was poorer. However, DM content could be artificially increased by appropriate harvest and storage techniques, and lastly by forced drying. Thus, the possibility of additional costs for drying should be taken into account, especially at second cut in DH systems and in SH-A. On the other hand, drying in the field would be possible at summer cuts in Italy because of the typical weather conditions.

The choice between different harvest and storage systems should be based on the conversion technology adopted and on the characteristics of the supply chain. In fact, moisture content does not negatively affect biomass conversion into biogas, but rather influences technology choices for the anaerobic digestion process [43]. The use of wet biomass has also been proposed in bioethanol plants, although research and economic analysis have generally emphasized dry storage. In fact, wet storage could reduce harvest costs, improve feedstock susceptibility to enzymatic hydrolysis, and integrate chemical or biological pretreatments [44]. Wet materials are typically stored in anaerobic silage conditions, in order to preserve both energy concentration and moisture. However, high DM content may reduce the availability of carbohydrates for lactic fermentation, and thus for ensiling. According to Smith et al. [45], giant reed from FC and SC-A showed the most suitable DM contents for ensiling (DM <50 %), while feedstock from SC-W and SH-S approached the threshold in both years. Compared with plants harvested once per year, less mature biomass obtained by double-cut management is typically more suitable for anaerobic digestion, as suggested by higher methane potentials reported for giant reed and switchgrass [35, 46].

Note that in our study the ash content of biomass from summer harvests and second cuts was about twofold that of biomass harvested once per year in winter, thus showing a clear decrease in feedstock quality for thermochemical conversion under double harvest systems. In line with our work, Monti et al. [32] observed that double-cut management caused the ash content to be increased both at first and second cuts compared with single-cut management in switchgrass. Results obtained on reed canary grass by Tahir et al. [19] were quite different, since second and single cuts harvested in autumn and winter were higher in ashes than first cuts harvested early in the season. In general, unless biomass quality is altered by external impurities (e.g., soil particles), the ash concentration in bioenergy crops is related to juvenility and increased leaf/stem ratio [25, 29]. Nonetheless, despite the high ash content and likely unfavorable ash properties, troublesome feedstock could be conveniently managed in combustion plants adopting specifically purposed technologies [23, 47]. Double cutting might be a reasonable option for thermochemical conversion, provided that its advantages (e.g., natural drying out of the feedstock in summer) are not outweighed by the disadvantages (e.g., reduced quality, higher costs). On the other hand, no adverse effects on anaerobic digestion are known, although a relative decrease in the organic fraction theoretically available for fermentation might be expected.

Conclusions

Giant reed has a significant potential to deliver high biomass yields for bioenergy production, and its biomass quality varies considerably according to harvest management. In our study, fundamental quality traits were affected by different harvest systems without significant effects on biomass yield over the considered years. A single delayed harvest improved biomass quality for thermochemical conversion, while in double harvest systems quality was affected by both first and second cut times. Results showed that giant reed could be exploited in different ways according to configuration and requirements of the bioenergy chains. Double-cut management could be a valuable option when biomass suitability for conversion is increased and the disadvantages from low dry matter and high ash content are not critical or can be overcome (e.g., anaerobic digestion). Furthermore, double harvest systems could provide a longer year-round supply of biomass to conversion plants and feedstock diversification, intended as the possibility to address biomasses obtained at different harvest times to different supply chains, according to their characteristics and to the time of the year in which they are obtained (e.g., first harvest for “dry” supply chains, second harvest for “wet” processes).

The feasibility of double harvest systems on giant reed should be considered with regard to the bioenergy pathways to be supplied, depending on feedstock quality, but also on long-term regrowth capacity, nutrient requirements, and economic aspects. In fact, the contribution of the second cut to the overall biomass yield should be considered from an economic point of view, since sustainability of the additional harvest costs could be hampered by low regrowth levels. Moreover, increased nutrient requirements can be expected, thus potentially leading to an intensification of the cropping system. Regrowth capability and overall productivity over time should also be further studied, since repeated cutting may result in the depletion of belowground reserves, thus leading to a reduced life span of the plantation.

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References

1. Karp A, Shield I (2008) Bioenergy from plants and the sustainable yield challenge. New Phytol 179:15–32
2. Zegada-Lizarazu W, Elbersen HW, Cosentino SL, Zatta A, Alexopoulou E, Monti A (2010) Agronomic aspects of future energy crops in Europe. Biofuels Bioprod Bioref 4:674–691
3. Angelini LG, Ceccarini L, Nassi o Di Nasso N, Bonari E (2009) Comparison of Arundo donax L and Miscanthus x giganteus in a long-term field experiment in Central Italy: analysis of productive characteristics and energy balance. Biomass Bioenergy 33:635–643
4. Monti A, Fazio S, Venturi G (2009) Cradle-to-farm gate life cycle assessment in perennial energy crops. Eur J Agron 31:77–84
5. Nassi o Di Nasso N, Roncucci N, Bonari E (2013) Seasonal dynamics of aboveground and belowground biomass and nutrient accumulation and remobilization in giant reed (*Arundo donax* L): a three-year study on marginal land. Bioenergy Res 6:725–736
6. Rossa B, Tüffers AV, Naidoo G, Von Willert DJ (1998) Arundo donax L (Poaceae)—a C3 species with unusually high photosynthetic capacity. Bot Acta 111:216–221
7. Lewandowski I, Scurlock JM, Lindvall E, Christou M (2003) The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass Bioenergy 25:335–361
8. Barney JN, DiTomaso JM (2008) Nonnative species and bioenergy: are we cultivating the next invader? Bioscience 58(1):64–70
9. Barney JN (2014) Bioenergy and invasive plants: quantifying and mitigating future risks. Invasive Plant Sci Manag 7:199–209
10. Boland JM (2006) The importance of layering in the rapid spread of *Arundo donax* (giant reed). Madrono 53(4):303–312

11. Virtue JG, Reynolds T, Malone J, Williams C. (2010). Managing the weed risk of cultivated *Arundo donax* L. Seventeenth Australasian Weeds Conference, Christchurch, New Zealand, pp. 176–179
12. 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 Crop Res 114:204–213
13. Nassi o Di Nasso N, Angelini LG, Bonari E (2010) Influence of fertilisation and harvest time on fuel quality of giant reed (*Arundo donax* L) in central Italy. Eur J Agron 32:219–227
14. Nassi o Di Nasso N, Roncucci N, Triana F, Tozzini C, Bonari E (2011) Seasonal nutrient dynamics and biomass quality of giant reed (Arundo donax L.) and miscanthus (Miscanthus x giganteus Greef et Deuter) as energy crops. Ital J Agron 6:152–158
15. Borin M, Barbera AC, Milani M, Molari G, Zimbone SM, Toscano A (2013) Biomass production and N balance of giant reed (*Arundo donax* L) under high water and N input in Mediterranean environments. Eur J Agron 51:117–119
16. Lewandowski I, Heinz A (2003) Delayed harvest of miscanthus—influences on biomass quantity and quality and environmental impacts of energy production. Eur J Agron 19:45–63
17. Adler PR, Sanderson MA, Boateng AA, Weimer PJ, Jung HJG (2006) Biomass yield and biofuel quality of switchgrass harvested in fall or spring. Agron J 98:1518–1525
18. Burvall J (1997) Influence of harvest time and soil type on fuel quality in reed canary grass (*Phalaris arundinacea* L). Biomass Bioenergy 12:149–154
19. Tahir MHN, Casler MD, Moore KJ, Brummer EC (2011) Biomass yield and quality of reed canarygrass under five harvest management systems for bioenergy production. Bioenergy Res 4:111–119
20. Kering MK, Butler TJ, Biermacher JT, Guretzky JA (2012) Biomass yield and nutrient removal rates of perennial grasses under nitrogen fertilization. Bioenergy Res 5:61–70
21. Smith R, Slater FM (2011) Mobilization of minerals and moisture loss during senescence of the energy crops *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea* in Wales, UK. Glob Chang Biol Bioenergy 3:148–157
22. Monti A, Di Virgilio N, Venturi G (2008) Mineral composition and ash content of six major energy crops. Biomass Bioenergy 32:216–223
23. Obernberger I, Brunner T, Bärnthaler G (2006) Chemical properties of solid biofuels—significance and impact. Biomass Bioenergy 30:973–982
24. Prochnow A, Heiermann M, Plöchl M, Linke B, Idler C, Amon T, Hobbs PJ (2009) Bioenergy from permanent grassland—a review: 1. Biogas. Bioresour Technol 100:4931–4944
25. Prochnow A, Heiermann M, Plöchl M, Amon T, Hobbs PJ (2009) Bioenergy from permanent grassland—a review: 2. Combustion. Bioresour Technol 100:4945–4954
26. Robson P, Mos M, Clifton-Brown J, Donnison I (2012) Phenotypic variation in senescence in Miscanthus: towards optimising biomass quality and quantity. Bioenergy Res 5:95–105
27. Amon T, Amon B, Kryvoruchko V, Machmüller A, Hopfner-Sixt K, Bodiroza V, Hrbek R, Friedel J, Potsch E, Wagentristl H, Schreiner M, Zollitsch W (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour Technol 98:3204–3212
28. Monlau F, Barakat A, Trably E, Dumas C, Steyer JP, Carrère H (2013) Lignocellulosic materials into biohydrogen and biomethane: impact of structural features and pretreatment. Crit Rev Environ Sci Technol 43:260–322
29. Nizami AS, Korres NE, Murphy JD (2009) Review of the integrated process for the production of grass biomethane. Environ Sci Technol 43:8496–8508
30. Guretzky JA, Biermacher JT, Cook BJ, Kering MK, Mosali J (2011) Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. Plant Soil 339:69–81
31. Fike JH, Parrish DJ, Wolf DD, Balasko JA, Green JT Jr, Rasnake M, Reynolds JH (2006) Switchgrass production for the upper southeastern USA: influence of cultivar and cutting frequency on biomass yields. Biomass Bioenergy 30:207–213
32. Monti A, Bezzi G, Pritoni G, Venturi G (2008) Long-term productivity of lowland and upland switchgrass cytotypes as affected by cutting frequency. Bioresour Technol 99:7425–7432
33. Grisso RD, McCullough D, Cundiff JS, Judd JD (2013) Harvest schedule to fill storage for year-round delivery of grasses to biorefinery. Biomass Bioenergy 55:331–338
34. Sharma KP, Kushwaha SPS, Gopal B (1998) A comparative study of stand structure and standing crops of two wetland species, *Arundo donax* and *Phragmites karka*, and primary production in *Arundo donax* with observations on the effect of clipping. Trop Ecol 39:3–14
35. Ragaglini G, Dragoni F, Simone M, Bonari E (2014) Suitability of giant reed (*Arundo donax* L) for anaerobic digestion: effect of harvest time and frequency on the biomethane yield potential. Bioresour Technol 152:107–115
36. Bates D, Maechler M, Bolker B (2012) Lme4: Linear mixed-effects models using S4 classes. http://cran.r-project.org/web/packages/lme4/index.html Accessed 12 Sep 2012
37. Tremblay A, Ransijn J (2013) Package 'LMERConvenienceFunctions'. A suite of functions to back-fit fixed effects and forward-fit random effects, as well as other miscellaneous functions. http://cran.r-project.org/web/packages/LMERConvenienceFunctions/index.html Accessed 12 Sep 2013
38. Bagnouls F, Gaussen H (1957) Les climats biologiques et leur classification. Ann Geogr 355:193–220
39. Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
40. Suzuki JI, Stuefer J (1999) On the ecological and evolutionary significance of storage in clonal plants. Plant Species Biol 14:11–17
41. Decruyenaere JG, Holt JS (2001) Seasonality of clonal propagation in giant reed. Weed Sci 49:760–767
42. Kavanova M, Gloser V (2005) The use of internal nitrogen stores in the rhizomatous grass *Calamagrostis epigejos* during regrowth after defoliation. Ann Bot 95:457–463
43. Deublein D, Steinhauser A (2011) Biogas from waste and renewable resources: an introduction. Wiley-VCH, Weinheim
44. Digman MF, Shinners KJ, Casler MD, Dien BS, Hatfield RD, Jung HJG, Muck RE, Weimer PJ (2010) Optimizing on-farm pretreatment of perennial grasses for fuel ethanol production. Bioresour Technol 101(14):5305–5314
45. Smith WA, Bonner IJ, Kenney KL, Wendt LM (2013) Practical considerations of moisture in baled biomass feedstocks. Biofuels 4:95–110
46. Massé D, Gilbert Y, Savoie P, Bélanger G, Parent G, Babineau D (2010) Methane yield from switchgrass harvested at different stages of development in Eastern Canada. Bioresour Technol 101:9536–9541
47. Picco D, Venturi G (2013) Experiences in north-east Italy: pellet combustion of common reed and other field crops in small power plants. Ital J Agron 8:40–44