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ORIGINAL RESEARCH

Biomass and methane yield of giant reed (*Arundo donax* L.) as affected by single and double annual harvest

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Abstract

The replacement of silage maize with giant reed as energy crop has been proposed as a mean for reducing the need of irrigation water as well as monetary and environmental costs of cultivation. Little is known about giant reed response to within-season harvesting, and its effect on the methane production in anaerobic digestion. The effect of three harvest schedules on yield, biomass composition and methane production of giant reed was evaluated at one site of the Po Valley, northern Italy, for three consecutive years. In a completely randomized block design with four replicates the treatments applied annually were: (i) double harvest 1: first cut at the end of June + second cut at the beginning of October (DH1); (ii) double harvest 2: first cut at the end of July + second cut at the beginning of October (DH2); (iii) single harvest at the beginning of October (SH). The crop stand was established in the year 2015 and treatments were repeatedly applied in the years 2016, 2017 and 2018. The SH treatment determined the highest average annual dry matter (DM) yield (59.0 Mg DM ha^{-1}). The DM yield for treatments with double harvest was significantly lower, that is, −30% for DH1 and −15% for DH2. In terms of specific methane yield there was little advantage in harvesting biomass twice during the growing season. The average specific methane yield varied substantially between years (i.e. from 144 to 233 ml CH_4 g^{-1} VS), and in every year the values tended to decrease with the ageing of harvested biomass. Methane yield per hectare, however, was driven by DM yield, thus SH also determined the highest average value (9110 m^3 CH_4-STP ha^{-1}). In conclusion, the single annual harvest at the end of the growing season is an ideal strategy for maximizing methane production from giant reed.

KEYWORDS

anaerobic digestion, Arundo, biogas, biomass composition, single and double harvest

1 | INTRODUCTION

Over the last two decades, Italy has witnessed a substantial proliferation of biogas plants. Most of these plants are concentrated in areas with intensive livestock activities and use silage maize (*Zea mays* L.) and/or livestock manure as feedstocks for the anaerobic digestion (AD; Carrosio, 2014). In such areas maize has been traditionally the prevalent crop,

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providing, in the form of grain and silage the nutritional base for both fattening and milking animals. Following the Nitrates Directive (EEC, 1991) concerning the protection of waters against pollution caused by nitrates from agricultural sources, stakeholders were forced to curb the environmental footprint of animal waste utilization. Yet, following the European Renewable Energy Directive (EC, 2009/28/EC, 2009), on the promotion of the use of energy from renewable sources, Italian policy provided monetary incentive to electricity produced from biogas plants (Carrosio, 2013). Owing to such incentive, electricity production from biogas had become the main economic purpose for many farms. This, in turn, re-shaped the organizational model of farms: silage maize was cultivated to provide biomass to the digester, while grain maize for animal feeding was purchased on the international market (Carrosio, 2013).

Maize is not only water demanding but also an intensive crop in terms of nutrient supply, weed and pest management and soil tillage. Hence, there is much scope to explore alternative crops as biomass feedstock that could be cultivated in rainfed conditions while reducing cultivation costs and environmental side effects (Corno et al., 2014). Giant reed (*Arundo donax* L.) is a non-food, tall, rhizomatous grass that produces a substantial amount of lignocellulosic biomass in Mediterranean climates (Lewandowski et al., 2003). From the standpoint of reducing agronomic inputs, this species possesses several key attributes (Ceotto & Di Candilo, 2010a, 2010b): (i) it is perennial, therefore eliminates the need of annual soil tillage; (ii) it can be cultivated without irrigation; (iii) it has efficient use of nitrogen, which is seasonally translocated from the above to below plant organs and vice-versa; (iv) it is not susceptible to pathogens and a strong competitor against weeds, thus eliminating the need of chemicals application. Giant reed is usually cultivated in rainfed conditions in northern Italy, thus it could replace maize whether irrigation water is unavailable or too expensive. Yet, this crop can be suitably cultivated even in marginal areas, unsuited for grain crop cultivation, owing not only to low fertility but also to environmental pollution and safety reasons (Ceotto & Di Candilo, 2010b). According to D'Imporzano et al. (2018) the high productivity of giant reed, combined to its low requirement of agronomic inputs, is key for minimizing the environmental impact of the crop. Moreover, giant reed exerts an effective soil nitrate removal, therefore reducing the risk of nitrate pollution (Ceotto et al., 2018). While giant reed has been largely investigated during the last decade, most of the studies focused on the crop harvested when the annual growth has ceased, notably in autumn or winter (Angelini et al., 2009; Borin et al., 2013; Cosentino et al., 2006; Monti & Zegada-Lizarazu, 2016). Little is known about the performances of the giant reed stand harvested during the growing season. This study was solicited by the stakeholders of the biogas sector, who raised these specific questions: how much biomass and methane yield can be obtained if the giant reed stand is harvested twice, instead of once, per growing season? In the case that giant reed is harvested in midsummer would crop regrowth be substantial?

A preliminary study of Ragaglini et al. (2014) provided encouraging results, but it was still limited to only one growing season. The following research questions were addressed:

(i) What is the best harvesting schedule for obtaining the highest giant reed biomass productivity?
(ii) In which period of the growing season is the giant reed biomass better suited as feedstock for anaerobic digestion?
(iii) Which is the best compromise between biomass quality and quantity to obtain the highest methane yield per hectare form giant reed?

To answer these questions a field experiment was carried out for 3 years comparing giant reed harvesting schedules.

2 | MATERIALS AND METHODS

2.1 | Site characterization

The experimental site is located in Anzola dell'Emilia (Bologna), in the alluvial plain of Po Valley, northern Italy (Lat. 44°32′N, Long. 11°11′E, 38 m a.s.l.). The soil of the site is a silty loam, classified as fine silty, mixed mesic Udifluventic Haplusteps (Soil Survey Staff, 2014). The main soil characteristics are reported in Table 1. The climate is temperate sub-continental, owing to the relatively long distance from the sea of about 200 km. The average annual air temperature for the site is 12.9°C, and the mean annual total rainfall is 755 mm. The location is characterized by a relatively shallow water table, usually fluctuating between 1.1 m depth in winter and about 2 m depth in summer. The presence of such a water table assures favourable production conditions for giant reed under rainfed cultivation.

2.2 | Agronomic experiment

The giant reed stand was established in June 2015. The preceding crop was winter barley (*Hordeum vulgare* L.), harvested at the end of May at the milky kernel stage. After the barley harvest, the soil was ploughed at 0.30 m depth and subsequently refined with a rotary harrow to allow furrows preparation and a favourable soil bed condition for stem cuttings.

The propagation technique proposed by Ceotto and Di Candilo (2010a) was used: shoot cuttings of about 1 m length, with several nodes bringing lateral branches, were laid down horizontally on furrows of 0.15 m depth, and then covered manually with the soil. Two parallel culms

TABLE 1 Main chemical and physical characteristics of the soil

	Soil layer depth							
	0–0.2 m		0.2–0.4 m		0.4–0.6 m		0.6–0.8 m	
Soil characteristics	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sand (2.0–0.05 mm) (g kg^{-1})	367	34.5	343	79.9	332	24.8	360	51.9
Silt (0.05–0.002 mm) (g kg^{-1})	400	28.3	405	48.3	423	23.9	432	61.8
Clay (<0.002 mm) (g kg^{-1})	233	27.4	252	37.6	245	15.1	208	18.0
pH in water	7.86	0.15	7.82	0.21	7.81	0.18	7.89	0.19
$CaCO_3$ total (g kg^{-1})	14.9	1.31	15.3	0.65	15.2	1.19	16.4	1.73
Walkley and Black SOC (g kg^{-1})	10.6	0.14	9.90	0.40	9.70	0.60	6.90	0.50
Kjeldahl N (g kg^{-1})	0.85	0.04	0.81	0.04	0.76	0.07	0.48	0.08
Olsen P (mg kg^{-1})	18.6	10.3	12.9	2.53	13.1	7.04	5.85	1.28
Exchangeable K (mg kg^{-1})	504	69.2	371	85.6	286	76.0	247	73.3

were placed on the bottom of the furrows, aiming to increase the plant density. The distance between the furrows was 0.75 m. The field area planted with giant reed was about 2900 m^2. The stem cuttings were harvested on a giant reed stand, with culms at their second years, located in the same experimental farm. The clone used for the experiment was originally from the nearby location of Budrio (Bologna). Stem cuttings were planted from 22 to 23 June 2015 and immediately irrigated with a volume of 35 mm, using a linear move sprinkler irrigation system covering all the surface of the field. To facilitate stem rooting and crop establishment, the same irrigation volume was applied weekly until the end of August 2015. Few days after the first irrigation, and prior to the emergence of the giant reed sprouts, newly emerged weeds were controlled by a non-selective herbicide (glyphosate). No fertilization was applied in the planting year 2015.

In the following three growing seasons the giant reed stand was fertilized annually with 250 kg nitrogen ha^{-1}, applied in the form of urea at the beginning of April. This fertilization rate was devised on the base of a previous 4-year study conducted in the same location. Such study indicated that giant reed possesses high agronomic efficiency of applied nitrogen (Ceotto et al., 2015) and the ability to reduce the risk of nitrate pollution dealing with nitrogen surplus. (Ceotto et al., 2018).

No further irrigation was applied. This high fertilization rate was provided with the intent of ensuring abundant nitrogen availability, resulting in non-limiting nutrient conditions to the crop. No fertilization was applied during the first growing season, during which the crop encountered its juvenile phase. The above-ground biomass produced during the first growing season was harvested during the winter.

The harvesting schedule experiment was conducted during three consecutive years, 2016, 2017 and 2018, corresponding respectively to the second, third and fourth year of the crop age respectively. The harvesting treatments applied annually were: (i) double harvest 1: first cut at the end of June + second cut at the beginning of October (DH1); (ii) double harvest 2: first cut at the end of July + second cut at the beginning of October (DH2); (iii) single harvest at the beginning of October (SH). A scheme of the harvesting treatments applied during the 3 years of experiment is reported in Table 2.

TABLE 2 Time scheme of harvesting treatments and the corresponding number of days since the crops started growing after the winter rest or the last seasonal cutting (DORG)

Year	Crop age	Treatments	Date of harvest	DORG (days)
2016	Second year	DH1_I	29 June	61
		DH2_I	28 July	90
		DH1_II	28 September	90
		DH2_II	28 September	61
		SH	28 September	152
2017	Third year	DH1_I	26 June	57
		DH2_I	1 August	93
		DH1_II	5 October	100
		DH2_II	5 October	64
		SH	5 October	158
2018	Fourth year	DH1_I	26 June	56
		DH2_I	30 July	90
		DH1_II	15 October	110
		DH2_II	15 October	76
		SH	15 October	168

The treatment DH1 was directly solicited by the stakeholders, because it provides substantial biomass yield while leaving a relatively long period ahead for regrowth. The treatment DH2 was stimulated by the peculiar eco-physiology of the crop: Ceotto et al. (2013), working at the same site,

indicated a linear crop growth until the end of July and a subsequent 'summer slump' for giant reed growth. Then a harvest was devised at the end of the linear growth period to assess whether a harvest might rejuvenate growth. The treatment SH can be regarded as the reference because giant reed is usually harvested once per year, either in autumn or winter. The experimental design was a completely randomized block with four replications. The size of the individual plot was 99 m^2, defined by 4.5 m width (i.e. six rows distant 0.75 m) and 22 m length. The rectangular shape was adopted to allow the use of a tractor-mounted scissors lawn mower for harvesting.

2.3 | Weather data

Weather data for temperature and precipitation were collated from a meteorological station located at 200 m from the giant reed stand. Climatic data were recorded electronically at 1 min intervals and values were integrated to daily data.

2.4 | Biomass measurements and chemical analyses

An area of about 9 m^2 was sampled at random on each individual plot at every harvest date to measure the biomass produced per unit area (i.e. 4 internal rows × 0.75 m = 3 m width × 3 m length). External rows were excluded from sampling to minimize the edge effect. The individual stems and leaves of each fresh biomass sub-sample were cut manually in pieces of about 3–5 cm length, by using a pruning scissor prior to oven drying and milling and sieving at 1 mm size. After fresh weight measurement, a sub-sample composed of 3–4 kg of entire shoots (including stems and their leaves) was oven-dried at 65°C until a constant weight was obtained. Shoot dry biomass was milled through a 1-mm sieve (Cutting mill SM 300, Retsch® mbH). For the determination of total C and N of harvested biomass samples, ground samples of 0.150 grams were loaded into tin foil cups and analysed with LECO Truspec® CHN Analyzer (LECO Corporation).

Oven-dried, milled and sieved biomass samples were used to determine total solids (TS), volatile solids (VS), ash and pH according to APHA (2017). In particular, total solids were determined gravimetrically by thermal treatment at 105°C at constant weight. Volatile solids were determined as the difference between TS and ashes. Ash were determined by incineration in a muffle furnace at 550°C for 10 h. The pH was determined after suspension, 2-h stirring and sedimentation of 1.1 g dry matter in 50 ml distilled water pH, by means of a Crimson Titromatic 1S pH metre. Fibre fractions (neutral detergent fibre, NDF; acid detergent fibre, ADF; and lignin, ADL) were determined on samples dried at 65°C at constant weight according to Van Soest et al. (1991). The hemicellulose content was estimated as the difference between NDF and ADF; cellulose as the difference between ADF and ADL. Total polyphenols were determined according to the Folin−Ciocalteu colorimetric assay (Singleton & Rossi, 1965) and expressed as mg tannic acid per g of dry matter.

2.5 | Anaerobic digestion experiment

Representative samples of dry milled biomass collected on each plot (oven-dried, milled and sieved at 1 mm as described above) were used as feedstocks in AD experiments. Field replications of each treatment were pooled before being used in AD. The pH was determined by a Crimson Titromatic 1S pH metre, using 1.1 g dry matter, suspended, stirred and sedimented in 50 ml distilled water.

Anaerobic digestion of pooled samples was carried out in mesophilic conditions (35°C), in 100 ml batch bioreactors (118.5 ml effective volume), with five replications (five treatments, five replications and 3 years, corresponding to 75 reactors in total). The inoculum was prepared according to Vasmara et al. (2015). AD was carried out according to Vasmara and Marchetti (2016). Following Chartrain and Zeikus (1986) and Wu et al. (1992), the reaction mixture included 1 g of giant reed VS, 50 ml of sterilized phosphate-buffered basal medium without energy sources (HM), and 5 ml of inoculum. The initial pH of the mixtures was, on average, 7.4 ± 0.1. Three reactors containing only inoculum and HM were also included as blanks.

Biogas volume and composition were determined for each reactor during the incubation period (3 months). The cumulated methane production was determined as the sum of the methane volume collected in the syringe and that accumulated in the headspace of the reactors and expressed in standard conditions (STP) of temperature (273.15 K) and pressure (101.3 kPa). The methane yield per hectare was calculated by estimating the VS content (Mg ha^{-1}) of the dry matter yield of individual plots (i.e. subtracting ashes from DM) and then by multiplying that value for the SMY of the treatment.

Methane concentration in the biogas was determined by means of a MicroGC Agilent 3000 gas chromatograph, equipped with two columns: Molsieve and Plot U; detector: TCD. Carrier gas: argon. No methane production was detected in the blank reactors, therefore no CH_4 subtraction to the methane production of the samples was needed. The incubation period was considered completed when there was no more biogas production in any of the reactors.

2.6 | Fitting a modified Gompertz equation for anaerobic digestion

The Gompertz model was originally developed to describe population growth evolution. It represents the situation in which the relative growth rate is an exponentially declining function of time (Zwietering et al., 1990).

The kinetic of methane production in a reactor can be conveniently represented by a modified Gompertz function (Díaz et al., 2011; Velázquez-Martí et al., 2018). Following Lay et al. (1997) a modified three-parameter Gompertz equation (Equation 1) was applied in this study to describe the dynamic of CH_4 production.

$$M_{(t)} = M_{max}\exp\left\{-\exp\left[\left(\frac{eR_{max}}{M_{max}}\right)(\lambda - t) + 1\right]\right\}, \quad (1)$$

where $M_{(t)}$ (ml CH_4-STP g^{-1} VS) is the cumulative amount of CH_4 produced, in standard conditions for temperature and pressure (STP); t (days) is the elapsed time; M_{max} (ml CH_4 g^{-1} VS) is the maximum cumulative CH_4 production; e (exp) is the Euler's number; R_{max} (ml CH_4 day^{-1} g^{-1} VS) is the maximum CH_4 daily production rate; and λ is the lag time duration (days), that is the time of microbial adaptation before exponential CH_4 production begins.

The time (days) necessary to reach M_{max} was estimated by calculating the M_{max}/R_{max} ratio and adding to the quotient the lag time duration.

Fitting of the Gompertz model to measurements was performed using the PROC NLIN of SAS (SAS Institute, 2015). The parameter values were estimated according to the Gauss–Newton method. The Gompertz function parameters M_{max}, R_{max} and λ were estimated for individual treatments, crop phases and replicates.

From the agronomic standpoint it is convenient to deal with the concept of Specific Methane Yield (SMY), which is the efficiency with which the VS of giant reed biomass are converted into methane. Because in this experiment 1 g VS of giant reed were introduced in each reactor, this entails that both M_{max} and R_{max} are here referred to 1 g of VS. Hence, M_{max} coincides with SMY.

2.7 | Statistical analysis

This field experiment is a classic case of repeated measures, taken over time on the same experimental units. Not only the same plots were repeatedly treated and sampled but also the same living giant reed plants. Our experimental unit was the individual plot, in which the giant reed stand received the harvesting treatments over three consecutive growing seasons. Overall, the plots of treatments DH1 and DH2 were harvested six times, while the plots of treatment SH were harvested three times.

The statistical analysis was performed with the PROC MIXED of SAS using the statement REPEATED to deal with the covariance of the experimental unit over time (SAS Institute, 2015). Such procedure has been specifically developed in the SAS system for managing repeated measures collected over time on the same experimental units. The normal distribution of data was verified by the Shapiro–Wilk test. The homogeneity of variances was evaluated by Bartlett's test (SAS Institute, 2015).

Different criteria were used to evaluate treatment effects for the agronomic and the AD trials, based on the relevant research questions underlying the aims of the two studies. In the case of the agronomic experiment the relevant question was how much biomass and methane yield can be obtained per hectare and per year, depending on whether such biomass has been harvested once or twice per growing season. Therefore, the values of the individual harvests (DH1_I and DH1_II; DH2_I and DH2_II) were added for comparing the treatments DH1, DH2 and SH.

Crop age was included in this analysis as a fixed effect. It is relevant, particularly from the agronomic standpoint, because it entails the evaluation of the effect of cumulative harvests. It is important to notice that at the end of the 3 years, the plots of treatments DH1 and DH2 had received six repeated harvests while the plots of treatment SH had received merely three harvests. Hence, a relevant agronomic question is whether double harvest repeated over time leads to yield decline. The factor crop age is interlaced with year-to-year weather variability, although such effect cannot be disentangled. Conversely, in the case of the AD laboratory trial, the relevant question was whether the biomass harvested during the growing season, is better suited to biogas production compared to SH at the end of the season. Hence, we here considered five independent treatments: DH1_I, DH1_II, DH2_I, DH2_II and SH.

The statistical significance between means was assessed with the statement LSMEANS (SAS Institute, 2015). The correlation matrices were calculated by the PROC CORR (SAS Institute, 2015). The Pearson correlation coefficient was considered.

3 | RESULTS

3.1 | Weather

In the years 2016 and 2018, corresponding to II and IV years of crop age, the cumulated precipitation for the period 1 January–31 October was +11% and +9% of the 30-year average for the location, which is 610 mm (Figure 1). In both years precipitation was well distributed during spring and summer, determining favourable conditions for giant reed. On the contrary, in the year 2017 the crop stand had received merely 326 mm of precipitation for the same interval, which corresponds to −47% compared with the 30-year average. The scarcity of precipitation

occurred in both spring and summer and was accompanied by unusually high air temperatures. A local network of piezometers indicated that the water table, which normally remains at about 2-m depth during the summer, dropped to the depth of 3 m during summer 2017. Therefore, the year 2017, corresponding to the third year of crop age, can be regarded as an unfavourable year for giant reed rainfed cultivation.

3.2 | Crop biomass yield

The statistical analysis of annual dry matter yield indicated that the factors crop age and treatment were both highly significant, while the interaction crop age × treatment was not significant (Table 3). The average yield measured in the second year of crop age (51.4 Mg DM ha^{-1}) was significantly higher than the mean yield measured in the third year (42.8 Mg DM ha^{-1}), while in the fourth year the yield increased by +10% compared to the second year, albeit such difference was not significant (Figure 2). Because the fourth year provided the highest yield, the lowest productivity measured in the third year cannot be attributed to repeated harvesting.

The SH treatment determined the highest annual DM yield (59.0 Mg ha^{-1}). The cumulated annual DM yield for treatments with double harvest were significantly lower, that is, −30% for DH1 and −15% for DH2. The individual average annual yield per harvest was respectively 24.7 and 16.5 Mg ha^{-1} for the first and the second harvest of DH1, 41.9 and 8.4 Mg ha^{-1} for the first and the second harvest of DH2. It is worth noting that the first harvest accounted for 60% of total average annual biomass yield for DH1 and 83% for DH2. Overall, our findings indicated that the more anticipated is the first harvest the lowest is the total annual DM yield.

Although the crop age × treatment interaction was not significant, additional insight is provided by the more accurate comparisons between individual means operated by the instruction LSMEANS of SAS (Littell et al., 1996, 1998). Two important points should be noted: (i) there was no significant difference between the SH yield of individual years (Figure 2); the yield reduction for both DH1 and DH2 compared to SH was particularly pronounced in the unfavourable year 2017 (Figure 2).

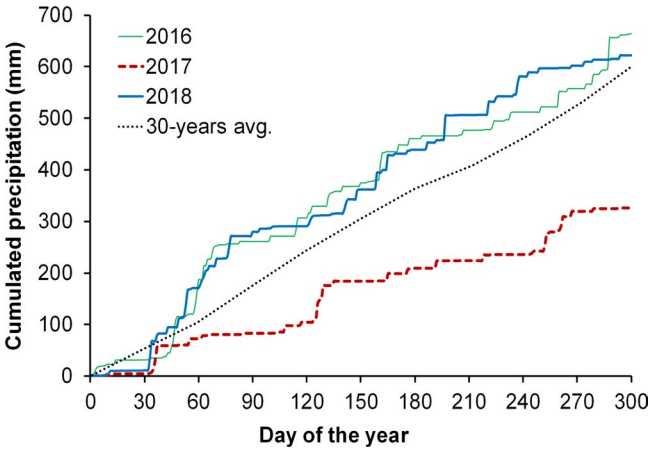


FIGURE 1 Accumulated precipitation from 1 January to 27 October for the years 2016, 2017 and 2018, corresponding respectively to the second, third and fourth year of the giant reed stand age

TABLE 3 Effects of crop age, treatment and interaction crop age × treatment on biomass dry matter yield and methane yield

Effect	Num DF	Den DF	*F* value	Pr > *F*
Biomass yield DM (Mg ha^{-1})				
Crop age	2	24	11.06	0.0004
Treatment	2	24	18.72	<.0001
Crop age × treatment	4	24	0.93	0.4654
Methane yield (m^3 ha^{-1})				
Crop age	2	24	71.9	<.0001
Treatment	2	24	9.71	0.0008
Crop age × treatment	4	24	0.55	0.6993

3.3 | Biomass composition

For most of the biomass composition traits, notably cellulose, lignin, ash, polyphenols, pH, carbon and nitrogen, we found significant differences not only regarding crop age and treatment but also for the interaction crop age × treatment (Table 4). In the case of hemicellulose only crop age and treatment effects were significant, but not their interaction.

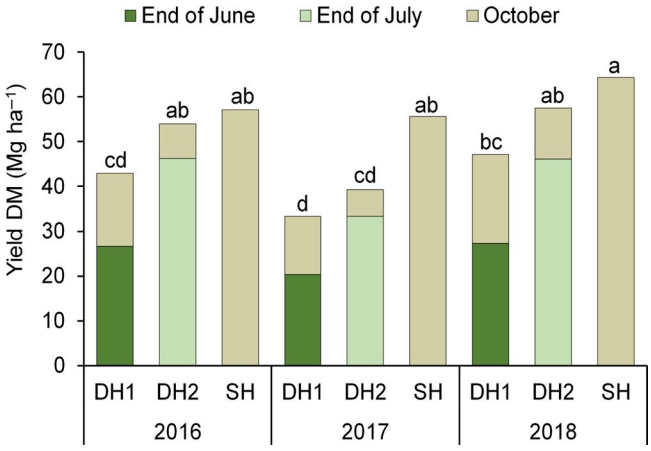


FIGURE 2 Patterns of annual dry matter yield for individual treatments throughout the 3 years of the experiment. Treatments sharing common letters are not significantly different a $p < 0.05$. Treatments were: DH1 = double harvest (June&October); DH2 = double harvest (July&October); SH = single harvest (October)

TABLE 4 Mean values of the qualitative biomass traits of harvested *Arundo donax*. Treatments: DH1 = double harvest (June+October); DH2 = double harvest (July+October); I = first cut; II =second cut; SH =single harvest (October). DORG is the number of days since the crops started growing after the winter rest or the last seasonal cutting; Hem =hemicellulose; Cell =cellulose; Lign =lignin; Poly =total polyphenols; C = total carbon; N = total nitrogen; Uppercase letters refer to years, lowercase letters refer to treatments within years. Means sharing commons letters within year are not significantly different for $p < 0.05$

Crop age	Treatment	DORG	Hem (% TS)	Cell (% TS)	Lign (% TS)	Ash (% TS)	Poly (% TS)	pH	C (% DW)	N (% DW)	C/N (% DW)
Second year	DH1_I	61	24.6ab	38.8bc	7.80b	8.32b	0.91a	5.34c	44.5c	1.72a	26.4d
	DH1_II	90	26.5a	39.1b	9.22a	8.38b	0.75c	5.61a	46.3b	0.99c	47.8b
	DH2_I	90	23.5b	41.2a	9.69a	6.59c	0.80b	5.36c	47.4a	1.31b	37.2c
	DH2_II	61	26.9a	37.1c	7.34b	9.74a	0.91a	5.52b	45.8b	1.95a	23.7d
	SH	152	22.9b	40.9a	10.9a	5.42d	0.75c	5.66a	47.7a	0.79c	70.0a
	Year avg.		24.9AB	39.4B	8.99A	7.69C	0.82A	5.50C	46.3A	1.35A	39.2B
Third year	DH1_I	57	25.2b	39.9a	8.11c	8.31c	0.94b	5.79b	45.7c	1.61b	28.5d
	DH1_II	100	24.3b	40.5a	8.46bc	10.5b	0.76c	5.65c	45.4c	1.18c	38.4c
	DH2_I	93	24.0b	40.3a	11.0a	6.83d	0.72d	5.85a	46.6b	0.95cd	49.7b
	DH2_II	64	30.5a	35.0b	5.93d	11.2a	0.98a	5.60d	45.3c	2.62a	18.2e
	SH	158	25.4b	39.5a	10.2ab	5.63e	0.72d	5.89a	48.2a	0.79d	64.6a
	Year avg.		25.9A	39.0B	8.74B	8.49A	0.82A	5.76B	46.2A	1.43A	39.9B
Fourth year	DH1_I	56	23.0a	41.0b	7.93cd	7.16c	0.77a	6.27a	43.4b	1.43a	30.4d
	DH1_II	110	23.9a	41.0b	8.80bc	8.81b	0.68c	6.04b	42.3c	0.81c	52.3b
	DH2_I	90	23.6a	42.1ab	9.33b	6.89c	0.72b	6.03b	43.6b	0.99bc	45.2c
	DH2_II	76	24.6a	40.4b	7.13d	11.0a	0.69bc	5.91c	41.0d	1.13b	37.2c
	SH	147	23.3a	43.5a	10.4a	5.69d	0.66c	5.87c	45.0a	0.72c	63.6a
	Year avg.		23.7B	41.6A	8.72B	7.91B	0.70B	6.02A	43.1B	1.02B	45.8A
	Crop age		**	***	***	***	***	***	***	***	***
	Treatment		***	***	***	***	***	***	***	***	***
	Crop age × Treat.		n.s.	*	***	***	***	***	***	***	***

*$p < 0.05$; **$p < 0.05$; ***$p < 0.001$; n.s., not significant.

Cellulose is a major component of the giant reed biomass, accounting for 35%–43% of the total solids (TS). As a general trend, the cellulose content tended to increase when hemicellulose content decreased, and vice versa. It should be noted that in the favourable season 2018 (the fourth year), the average cellulose content (41.6) was statistically higher than in the other years (+5% and +7% higher compared to second and third year).

Regarding lignin, in all the years the content tended to raise with increasing number of DORG. In the favourable fourth year the lignin content was significantly lower than the second year, but very close to the third year.

As far as ash is concerned, in the 3 years the content was significantly higher for the juvenile biomass harvesting, associated with low DORG values, compared to the late stage harvesting SH, characterized by a high DORG value. In the favourable fourth year, the ash content was significantly lower than in the third year, but higher than in the second year.

Polyphenols are a minor component of the giant reed biomass, accounting less than 1% of TS. In all the years polyphenol content was higher in the early harvesting with low DORG and tended to decrease with increasing DORG. Interestingly, in the advantageous fourth year polyphenols were significantly lower than both in the second and third year (−14.4%).

The carbon content tended to increase with the DORG. In the propitious fourth year the average carbon content was lower than in the other years. (−7%).

The nitrogen content progressively diluted with increasing DORG. Therefore, it followed a reverse trend compared to carbon. This trend was quite evident in each of the 3 years. However, in the most fortunate season (fourth year) the average nitrogen content was much lower than in the second and third year (−25% and −29% respectively). Consequently, the average C to N ratio, that was about 40 in both the second and the third year, raised to about 46 in the fourth year.

Overall, cellulose, lignin and carbon were positively correlated with DORG in all years of this study; in the same period ash, polyphenols and nitrogen were negatively correlated with DORG (Table 6).

3.4 | Anaerobic digestion

With regards to AD experiment, the statistical analysis performed for the three Gompertz parameters indicated significant effect for the crop age for M_{max}, R_{max} and **λ**, for treatments in case of M_{max} and **λ**, and no significant interaction crop age × treatment (Table 5). Although such interaction was not significant, the comparison between individual means of the treatments within individual years, operated by the instruction LSMEANS of SAS (Littell, et al., 1996, 1998) is reported in Table S1.

As far as treatments are regarded, we remind here that the first and second harvest of treatments DH1 and DH2 were here considered as separated treatments (i.e. DH1_I, DH1_II, DH2_I and DH2_II), because the purpose of AD experiment was to assess in which period of the growing season the giant reed biomass is best suited as feedstock for AD.

Overall, the effect of the age was much more pronounced than that of the treatment. In the case of M_{max} the highest value (233 ml CH_4 g^{-1} VS) was recorded in the fourth year and it was also accompanied by the highest value of R_{max} and the lowest value of λ (i.e. a shorter duration of the lag phase). Compared to the fourth year, the M_{max} was merely 66% in the second year and 62% in the third year. In the case of R_{max}, compared to the highest value of the fourth year (7.88 ml CH_4 day^{-1} g^{-1} VS), the values observed for the second and third year were 73% and 67% respectively. An opposite trend was observed for the lag phase duration **λ**, the highest value of 2.21 days was obtained in the second year, an almost identical value in the third year, and only 58% of the highest value on the fourth year.

As far as the treatments are concerned, significantly different M_{max} values were observed between the reference treatment single harvest (SH) and some combinations of early harvesting. The harvest of juvenile shoots (i.e. DH1_I and DH2_II), resulted in significantly higher M_{max} (+11% and +8% respectively) compared to SH, composed by shoots grown across the entire growing season. The remaining treatments DH1_II and DH2_I, composed by shoots of intermediate age, were not significantly different from the aforementioned treatments. The R_{max} values were not significantly different between the five treatments. (Table 5). The highest λ values were observed for the second cut of DH2 (DH2_II) and the lowest for SH. Overall, λ tended to decrease when DORG increased.

The general rule that can be inferred from our data is that giant reed juvenile biomass tends to determine both the highest final value of M_{max} and the longest lag phase. Nevertheless, the differences in the lag phase duration have been irrelevant since no differences were detected in the time to reach M_{max} (30 ± 1 days, on average) in all reactors.

Additional insight can be gained from the correlation matrices reported for the individual years between the three Gompertz parameters, the crop development, quantified by the variable DORG, and the components of the dry matter biomass (Table 6). M_{max} tended to be negatively correlated with DORG in all years, but statistical significance arose only in the fourth year. A significant negative correlation between the DORG and R_{max} was observed in the fourth year, but not in the other years. A negative correlation between **λ** and the DORG was statistically significant in all the 3 years. Accordingly, the lag time reduced when DORG increased.

TABLE 5 Mean values of the three parameters of the modified Gompertz function for anaerobic digestion for crop phase and treatments. The statistical significance for each factor based on analysis of variance is reported. Means sharing commons letters within year are not significantly different at $p < 0.05$

	M_{max} (ml CH_4 g^{-1} VS)	R_{max} (ml CH_4 d^{-1} g^{-1} VS)	λ (days)
Crop age			
Second year	153b	5.79b	2.21a
Third year	144b	5.28b	2.17a
Fourth year	233a	7.88a	1.28b
Treatment			
DH1_I	185a	6.66a	2.31ab
DH1_II	175ab	6.02a	1.78b
DH2_I	176ab	6.55a	1.63bc
DH2_II	180a	6.48a	3.03a
SH	167b	5.87a	0.70c
Crop age	***	***	*
Treatment	*	n.s.	***
Crop age × Treatment	n.s.	n.s.	n.s.

Abbreviations: **λ**, lag phase duration; M_{max}, maximum cumulative CH_4 production; R_{max}, daily rate of CH_4 accumulation.

*$p < 0.05$; ***$p < 0.001$; n.s., not significant.

3.5 | Methane yield per hectare

The statistical analysis of methane yield per hectare indicated that the factors crop age and treatment were highly significant while the interaction crop phase × treatment was not significant (Table 3). While the general patterns of methane yield retrace the ones of dry matter yield, differences between crop age were exacerbated because the fourth year (i.e. 2018) was characterized

TABLE 6 Pearson correlation coefficients for the three parameters of the modified Gompertz function, the biomass age and the qualitative biomass traits of giant red biomass

	R_{max}	λ	DORG	Hem	Cell	Lign	Ash	Poly	pH	C	N
Second year											
M_{max}	0.05	0.11	−0.25	0.03	−0.03	−0.17	0.11	0.16	−0.31	−0.23	0.16
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R_{max}		0.54	−0.10	−0.06	0.05	−0.05	−0.01	0.06	−0.20	−0.13	0.04
		**	ns	ns	ns	ns	ns	ns	ns	ns	ns
λ			−0.45	0.30	−0.35	−0.45	0.40	0.40	−0.29	−0.39	0.42
			*	ns	ns	*	*	*	ns	ns	*
DORG				−0.66	0.71	0.93	−0.87	−0.79	0.65	0.80	−0.87
				**	***	***	***	***	**	***	***
Third year											
M_{max}	0.27	−0.29	−0.36	0.21	−0.21	−0.25	0.18	0.35	−0.16	−0.25	0.31
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R_{max}		0.00	−0.08	−0.22	0.20	0.21	−0.12	−0.13	0.15	0.01	−0.15
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
λ			−0.43	0.52	−0.52	−0.50	0.42	0.50	−0.43	−0.39	0.57
			*	**	**	*	*	*	*	ns	**
DORG				−0.35	0.34	0.62	−0.64	−0.78	0.56	0.86	−0.72
				ns	ns	**	**	***	**	***	***
Fourth year											
M_{max}	0.76	0.21	−0.46	0.05	−0.47	−0.47	0.29	0.36	0.37	−0.27	0.45
	***	ns	*	ns	*	*	ns	ns	ns	ns	*
R_{max}		0.10	−0.43	0.21	−0.34	−0.44	0.32	0.25	0.09	−0.30	0.40
		ns	*	ns	ns	*	ns	ns	ns	ns	ns
λ			−0.64	0.40	−0.79	−0.76	0.67	0.34	0.32	−0.65	0.52
			**	*	***	***	**	ns	ns	**	**
DORG				−0.05	0.78	0.84	−0.44	−0.84	−0.70	0.41	−0.94
				ns	***	***	*	***	***	*	***

Note: DORG is the number of days since the crops started growing after the winter rest or the seasonal cutting.

Abbreviations: C, total carbon; Cell, cellulose; DW, dry weight at 65°C; Hem, hemicellulose; Lign, lignin; M_{max}, maximum cumulative CH_4 production; N, total nitrogen. TS, total solids at 105°C; Poly, total polyphenols; R_{max}, maximum daily rate of CH_4 accumulation; λ, lag phase duration.

*$p < 0.05$; **$p < 0.05$; ***$p < 0.001$; n.s., not significant.

by the highest SMY (i.e. M_{max}) combined with the highest biomass yield. As far treatments are concerned, the average annual methane yield of SH was the highest (9110 m^3 CH_4-STP ha^{-1}), the result for DH2 was not significantly lower (8121 m^3 CH_4-STP ha^{-1}), while the one for DH1 (6767 m^3 CH_4-STP ha^{-1}) was significantly lower than both the other treatments. It is worth noting that the first harvest provided 74% of total methane in the case of DH1 and 89% in the case of DH2. Despite of the fact that interaction crop age × treatments was not significant, additional insight can be gained by the comparison of individual means operated by the LSMEANS. Notably, it is worth noting that in the favourable year 2018, even the methane yield of the treatment DH1 significantly exceeded all treatments of the two precedent years (Figure 3).

As far as average annual treatment are regarded, compared to the methane yield of the second year (7036 m^3 CH_4-STP ha^{-1}), the result was −23% for the third year (5390 m^3 CH_4-STP ha^{-1}), and +64% for the fourth year (11,571 m^3 CH_4-STP ha^{-1}, Figure 3).

4 | DISCUSSION

4.1 | Dry matter yield

As far as the effect of crop age is concerned, the plausible explanation for the lowest yield measured in the third year lies on the unfavourable precipitation pattern occurred in the year

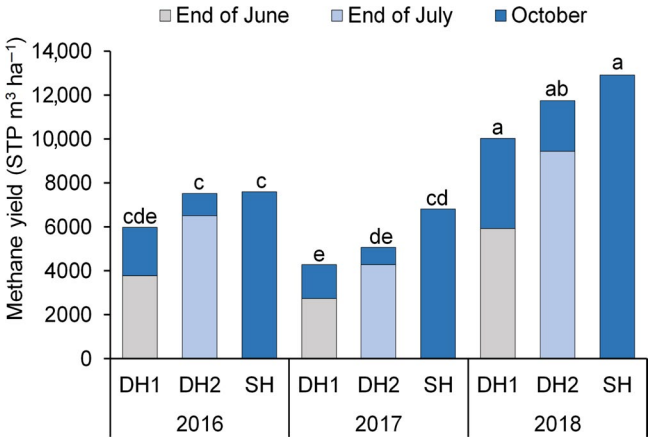


FIGURE 3 Patterns of annual CH_4 yield for individual treatments of the 3 years of the study. Treatments sharing common letters are not significantly different a $p < 0.05$. Treatments were: DH1 = double harvest (June&October); DH2 = double harvest (July&October); SH = single harvest (October)

2017 (Figure 1). In fact, despite to the repeated harvesting of the precedent years, the crop provided the highest yield in the favourable season 2018.

As far as the effects of single and double harvest are concerned, our findings are in contrast, but not in contradiction, with Ragaglini et al. (2014). Working in Tuscany, central Italy, these authors reported that double harvest (i.e. 35–40 Mg DM ha^{-1}) equaled that obtained by a SH at the end of the season (i.e. 38 Mg DM ha^{-1}). These authors explored cutting dates in the period from 21 June to 18 October, which is close to the time span considered in this study (i.e. 26 June–15 October). Because their data refer to the year 2011, and to a giant reed stand established in 2007, it is plausible that the rhizome reserves accumulated in the first 4 years of crop stand were strong enough to withstand a double harvest without reduction in the seasonal yield. The current study was carried out on a crop stand that was still on its juvenile phase and received the treatments for three consecutive years (i.e. second, third and fourth year of crop stand).

From the eco-physiological standpoint, the yield reduction determined by treatment DH1, with first cutting at the end of June, is supported by several concurrent circumstances. According to Ceotto et al. (2013) giant reed at the end of June has a substantial canopy cover, and this is combined with a high incoming solar radiation, due to the length of the day near the summer solstice. Hence, removing the whole canopy cover during the period with the highest solar irradiation is, in principle, a bad deal. Moreover, these authors, working at the same location, reported a steady daily growth rate for giant reed from early May until the beginning of August. Aiming to exploit thoroughly the linear phase of biomass accumulation across the season, the treatment DH2 (with the first harvest at the end of July) is certainly more appropriate. Indeed, for both treatments DH1 and DH2 the regrowth following the first cutting was not substantial enough to reach the yield obtained with SH and thus to justify two cuttings across the growing season.

The average annual DM yield observed for the reference treatment SH (59.0 Mg DM ha^{-1}) was higher compared to a previous study conducted on an abundantly fertilized giant reed stand in the same location. In fact, Ceotto et al. (2015) reported an average yield of 37.3 Mg DM ha^{-1} for a 5-year fertilization treatment consisting of 20 mm (i.e. 200 m^3 ha^{-1}) of cattle slurry per year. This is a 5-year average including one unfavourable year of poor cumulated precipitation. Because both experiments were based on the same clone, the most relevant difference consisted of timing of harvest: while in the cattle slurry trial biomass has been harvested during the winter, in the harvesting schedule experiment the SH treatment was harvested in October. A possible explanation could be that in October the translocation of dry matter into belowground organs, notably rhizomes, is still ongoing. This hypothesis is corroborated by a third study conducted at the location, in which biomass has been sampled throughout the growing seasons and biomass yield of about 60 Mg DM ha^{-1} was reported (Ceotto et al., 2013). In addition, Borin et al. (2013) reported a progressively declining yields of 74, 66 and 65 Mg DM ha^{-1} with autumn, mid-winter and late-winter, respectively, for giant reed growing under abundant nitrogen and water availability in northern Italy. It is also worth noting that the yield observed in this study is almost half of the highest ever giant reed yield (i.e. 125 Mg DM ha^{-1}) reported by Idris et al. (2012) working in Australia with experimental subsurface flow, gravel-based constructed wetlands receiving untreated recirculating aquaculture system wastewater.

In the last few years, a considerable amount of research has been devoted on evaluating the perennial grass Miscanthus (Miscanthus spp.) as feedstock for AD in substitution to maize (Kam et al., 2020; Kiesel & Lewandowski, 2017; Mangold et al., 2019; Ruf et al., 2017; Schmidt et al., 2018). Whittaker et al. (2016) pointed out that Miscanthus replacing maize is justified by the environmental benefits that may be achieved rather than simply the SMY, and by the type land which is available for it to be grown. All of these studies, however, were conducted in Central Europe and United Kingdom. Under the environmental conditions of Northern and Central Italy, giant reed appears to be better suited than Miscanthus due to its higher productivity. A medium-term experiment conducted in our experimental site involved a direct comparison between the two companion species (Di Candilo et al., 2008): the average yield for the period 2002–2008 was 39.6 Mg ha^{-1} for giant reed and 25.2 Mg ha^{-1} for Miscanthus. A lower but still substantial yield gap was observed on a long-term experiment conducted in Central Italy (Angelini et al.,

2009): the 12-year average yield was 37.7 Mg ha^{-1} for giant reed and 28.7 Mg ha^{-1} for Miscanthus.

4.2 | Biomass composition

To our knowledge, this is the first study exploring qualitative and quantitative responses of giant reed to harvesting treatments in three subsequent years. In the case of treatments combinations growing after winter and not considering regrowth, our observations are in good agreement with the findings of Nassi o Di Nasso et al. (2011). Working in Tuscany, central Italy, these authors reported the composition pattern of cellulose, hemicellulose and lignin for the biomass of a 7-year-old giant reed stand. In the period from July to October the cellulose content remained steady around 40%, the hemicellulose around 25% and the lignin content around 8%. While the order of magnitude of these contents is consistent with our observations, in this study the cellulose content increased from June to October in 2 of the 3 years, and the lignin content increased during the growing season in all the years.

The substantial decrease in ashes content during the period from June to October that we observed in this study is consistent with Monti et al. (2008). These authors reported a very high ashes content for giant reed leaves (11.3%) and relatively low ashes content for giant reed stems (3.2%). According to Ceotto et al. (2013) the fraction of leaves on total biomass decreases progressively in the period from June to October, hence it is plausible that the observed decrease in ashes can be attributed to the increasingly higher fraction of stems on total above-ground biomass at the end of the growing season.

Zegada-Lizarazu et al. (2020), working close to our location, reported a cellulose content of 41%–42% for giant reed clone obtained via mutagenesis. These contents were much higher than local clones used as a reference (36%–38%). Our study indicates that such supposedly high cellulose contents were not out of reach. In fact, using a local clone we obtained a maximum cellulose of content of 43.5% for the treatment SH in the favourable season 2018. Excluding the double cutting combination treatments which had an obvious influence of cellulose content, the SH cellulose content ranged from 39.0 to 43.5, suggesting that year-to-year variability can play a substantial role on determining the biomass composition.

4.3 | Methane production and biomass composition

We recall here that since in each reactor 1 g VS of giant reed was present, the M_{max} value corresponds to the quantity of methane that can be produced per unit of VS (CH_4 g^{-1} VS) which represents the specific methane yield (SMY). The average SMY values obtained in the 3 years of experiment (153, 144 and 233 ml CH_4 g^{-1} VS) fall within the range reported for this crop in previous studies. In fact, several authors reported average methane yields between 144 and 234 ml CH_4 g^{-1} VS from giant reed (De Girolamo et al., 2014; Jiang et al., 2016; Liu et al., 2015; Shilpi et al., 2019; Yang & Li, 2014). The high year-to-year SMY variability is a fact that previous studies have not highlighted since they referred to biomass harvested on a single year. This result, however, should not be surprising because the AD process and therefore the SMY value may be affected by the compositional quality of the biomass subjected to AD and, as mentioned in the results section, crop age has significantly influenced all the compositional parameters directly involved in the AD process. Recently, Shilpi et al. (2019) reported that by changing the irrigation method from tap water to abattoir wastewater, the biomass yield of giant reed has almost tripled and, at the same time, the SMY value has gone from 146 to 234 ml CH_4 g^{-1} VS, showing that favourable conditions to plant growth can positively affect CH_4 yield. This is consistent with this study, in which the favourable year 2018 not only increased biomass yields but also raised the cellulose content of biomass (Table 4). According to Li et al. (2018), among all the biomass components, cellulose is the one that exerts a positive influence on CH_4 production. This is confirmed in our study, in fact in the fourth year we observed a rise in cellulose content associated with an increase in the average SMY value.

In all years SMY tended to decrease with the increase in the intra-annual biomass ageing (DORG) of harvested biomass. This result should not be surprising because as the DORG increases, the amount of lignin present in the plant tissue increases (Table 4). It is known, indeed, that AD is negatively affected by the lignin content (Marchetti et al., 2016). Moreover, the cuts with lower DORG are characterized by an average C/N of 27 (Table 5) which fell within the range considered optimal for AD (20–30; Hagos et al., 2017). Since the carbon to nitrogen ratio is considered an important parameter for the AD process (Rabii et al., 2019), it is not surprising that low lignin content and an optimal C/N allowed younger shoots to reach the highest values of SMY.

Previously, Ragaglini et al. (2014) have evaluated the effect of double harvest on CH_4 production from giant reed. These authors reported that the double harvest increased CH_4 yield up to 68% compared to the SH (258 ml CH_4 g^{-1} VS). In particular, the first cut showed a SMY of 320 ml CH_4 g^{-1} VS whereas the second cut reached 381 ml CH_4 g^{-1} VS. This pattern disagrees with SMY values obtained in this study, since no differences between first and second cuttings as well as a minor CH_4 differences with the SH (i.e. 10%, on average) were found. This result, however, is in agreement with the biomass composition data:

the differences in biomass composition between DH1, DH2 and SH were very limited, especially in the components that are most involved in the CH_4 production in AD as cellulose and hemicellulose (Table 4); therefore, a substantial difference between DH1, DH2, or SH in terms of CH_4 production was not expected.

As far as the other parameters of the AD are concerned, the maximum CH_4 production rate (R_{max}) did not show significant differences between the cuts; this can be explained by the relatively homogeneous biomass composition for the different harvests. Hemicellulose is the fibre fraction that could influence the R_{max} value as it is rapidly hydrolysed and transformed into organic acids suitable for methanogenesis; in contrast, an excess of hemicellulose could lead to rapid acidification with consequent lowering of R_{max} (Li et al., 2018). In other plant species, high hemicellulose content characterizes the biomass in its juvenile stage (Lachowicz et al., 2019; Surendra & Khanal, 2015); in contrast, in this study hemicellulose significantly decreased with DORG only in 1 of the 3 years (Tables 4 and 6). This could explain the lack of differences between the cuts in terms of R_{max}. As far as λ is concerned, it was longer for biomass harvested the juvenile stage. Single harvest, indeed, showed an extremely low λ value (0.7 days, on average) compared to 2.67 days, on average, referred to plants in a very juvenile stage (i.e. DH1_I and DH2_II). This could be associated with either the amount of ash and extractives, as shown in Table 4, or the polyphenols abundancy in DH1_I and DH2_II. Recently, polyphenols have been linked to a delay in the start of the AD process (Vasmara & Marchetti, 2018). However, both the double harvests DH1 and DH2 had SMY values of 180 and 178 ml CH_4 g^{-1} VS, on average, which are significantly higher than that obtainable from SH (167 ml CH_4 g^{-1} VS), albeit no further beneficial effects on the AD process were revealed. Because DH1 and DH2 slightly increased SMY values compared to SH, the biomass yield per hectare mostly influenced the methane yield per hectare. The CH_4 yield per hectare was higher for SH compared to DH1 and DH2. On the basis of the results obtained, SH determined the best compromise between SMY and methane yield per hectare, while DH2 and DH1 slightly increased the SMY value but decreased biomass yield, and consequently lowered methane yield. Our findings are in contrast, but not in contradiction with Ragaglini et al. (2014) who reported that a double harvest strategy can increase methane yield per hectare by 20%–25%. However, the conditions in which the study of these authors was conducted should be considered in comparing results: the double harvest was applied in one single year, to a crop at its fourth year of age. It is likely that the rhizomes reserve of such mature crop was abundant enough to sustain vigorous regrowth in case of double cuttings. On the contrary, in this study the crop received the double harvest for three successive years, starting from the second year of age, and having presumably poorer rhizome reserves at disposal to counteract the within-season above-ground biomass removal.

As far as year-to-year variability is concerned, our outcomes indicate that lignin content itself does not explain the large variability of both M_{max} and R_{max} observed across the 3 years of study. In fact, the suitability of a biomass to provide a high methane yield appears to be the overall result of the contribution of several compounds. We recall here that the biomass of the advantageous fourth year was characterized by low nitrogen, low polyphenols and higher cellulose content compared to the previous 2 years. Because the lowest M_{max} was observed in the unfavourable dry season 2017 (third year) and the highest M_{max} in the subsequent favourable rainy season 2018 (fourth year), it is likely that the biomass suitability to be converted in methane took advantage of non-limiting production conditions encountered by the crop.

Overall, our results are consistent with the ones reported for Miscanthus by Kiesel and Lewandowski (2017). Working in Germany, these authors, indicated that early cuttings are unsustainable despite their higher SMY values, and reported a methane yield of about 6000 m^3 per hectare for the harvest in October, within the range of the methane productivity of silage maize.

5 | CONCLUSIONS

Several straightforward conclusions can be drawn from our study:

(i) The SH at the end of the growing season appears as the superior treatment in terms of both attainable biomass and methane yield per hectare, compared to double harvest. Yet, double harvest entails higher costs as well;
(ii) in terms of specific methane yield there is little advantage in harvesting biomass twice during the growing season, because this implies an extended period of the lag phase of microbial activity;
(iii) A strong year-to-year variability of giant reed performance can be expected, not only in terms of yield but also for biomass composition and specific methane yield. Then, a reliable unique value of specific methane yield for this species can hardly be indicated.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Angelini, L. G., 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 and Bioenergy*, *33*, 635–643. https://doi.org/10.1016/j.biombioe.2008.10.005

APHA. (2017). In E. W. Rice, R. B. Baird, & A. B. Eaton (eds), *Standard methods for the examination of water and wastewater* (23rd ed.). Author. https://www.awwa.org/Store/Product-Details/productId/65266295

Borin, M., Barbera, A. C., Milani, M., Molari, G., Zimbone, S. M., & Toscano, A. (2013). Biomass production and N balance of giant reed (*Arundo donax* L.) under high water and N input in Mediterranean environments. *European Journal of Agronomy*, *51*, 117–119. https://doi.org/10.1016/j.eja.2013.07.005

Carrosio, G. (2013). Energy production from biogas in the Italian countryside: Policies and organizational models. *Energy Policy*, *63*, 3–9. https://doi.org/10.1016/j.enpol.2013.08.072

Carrosio, G. (2014). Energy production from biogas in the Italian countryside: Modernization vs. repeasantization. *Biomass and Bioenergy*, *70*, 141–148. https://doi.org/10.1016/j.biombioe.2014.09.002

Ceotto, E., Castelli, F., Moschella, A., Diozzi, M., & Di Candilo, M. (2015). Cattle slurry fertilization to giant reed (*Arundo donax* L.): biomass yield and nitrogen use efficiency. *BioEnergy Research*, *8*(3), 1252–1262. https://doi.org/10.1007/s12155-015-9577-2

Ceotto, E., & Di Candilo, M. (2010a). Shoot cuttings propagation of giant reed (*Arundo donax* L.) in water and moist soil: the path forward? *Biomass and Bioenergy*, *34*, 1614–1623. https://doi.org/10.1016/j.biombioe.2010.06.002

Ceotto, E., & Di Candilo, M. (2010b). Sustainable bioenergy production, land and nitrogen use. In E. Lichtfouse (ed.), *Biodiversity, biofuels, agroforestry and conservation agriculture, sustainable agriculture reviews* (Vol. *5*, pp. 101–122). https://doi.org/10.1007/978-90-481-9513-8_3

Ceotto, E., Di Candilo, M., Castelli, F., Badeck, F. W., Rizza, F., Soave, C., Volta, A., Villani, G., & Marletto, V. (2013). Comparing solar radiation interception and use efficiency for the energy crops giant reed (*Arundo donax* L.) and sweet sorghum (*Sorghum bicolor* L. Moench). *Field Crops Research*, *149*, 159–166. https://doi.org/10.1016/j.fcr.2013.05.002

Ceotto, E., Marchetti, R., & Castelli, F. (2018). Residual soil nitrate as affected by giant reed cultivation and cattle slurry fertilisation. *Italian Journal of Agronomy*, *13*, 317–323. https://doi.org/10.4081/ija.2018.1264

Chartrain, M., & Zeikus, J. G. (1986). Microbial ecophysiology of whey biomethanation: Characterization of bacterial trophic populations and prevalent species in continuous culture. *Applied and Environmental Microbiology*, *51*(1), 188–196. https://doi.org/10.1128/AEM.51.1.188-196.1986

Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E., & Peck, M. W. (1993). Biochemical methane potential of biomass and waste feedstocks. *Biomass and Bioenergy*, *5*, 95–111. https://doi.org/10.1016/0961-9534(93)90010-2

Corno, L., Pilu, R., & Adani, F. (2014). *Arundo donax* L.: A non-food crop for bioenergy and bio-compound production. *Biotechnology Advances*, *32*, 1535–1549. https://doi.org/10.1016/j.biotechadv.2014.10.006

Cosentino, S. L., Copani, V., Agosta, G. M. D., Sanzone, E., & Mantineo, M. (2006). First results on evaluation of *Arundo donax* L. clones collected in Southern Italy. *Industrial Crops and Products*, *23*, 212–222. https://doi.org/10.1016/j.indcrop.2005.06.004

D'Imporzano, G., Pilu, R., Corno, L., & Adani, F. (2018). *Arundo donax* L. can substitute traditional energy crops for more efficient, environmentally-friendly production of biogas: A Life Cycle Assessment approach. *Bioresource Technology*, *267*, 249–256. https://doi.org/10.1016/j.biortech.2018.07.053

De Girolamo, G., Bertin, L., Capecchi, L., Ciavatta, C., & Barbanti, L. (2014). Mild alkaline pre-treatments loosen fibre structure enhancing methane production from biomass crops and residues. *Biomass and Bioenergy*, *71*, 318–329. https://doi.org/10.1016/j.biombioe.2014.09.025

Di Candilo, M., Ceotto, E., & Diozzi, M. (2008). Comparison of 7 ligno-cellulosic biomass feedstock species: 6-years results in the Low Po Valley. In: Rossi Pisa P. (ed.) 10th Congress of the European Society of Agronomy, Bologna, Multi-functional Agriculture, Agriculture as a Resource for Energy and Environmental Preservation. *Italian Journal of Agronomy/Rivista di Agronomia*, *3*(3 suppl.), 481–482.

Díaz, I., Donoso-Bravo, A., & Fdz-Polanco, M. (2011). Effect of microaerobic conditions on the degradation kinetics of cellulose. *Bioresource Technology*, *102*, 10139–10142. https://doi.org/10.1016/j.biortech.2011.07.096

EC, Directive 2009/28/EC. (2009). On the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. *The Official Journal of the European Union*, *5*. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009L0028

EEC (1991). Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31991L0676

Hagos, K., Zong, J., Li, D., Liu, C., & Lu, X. (2017). Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renewable and Sustainable Energy Reviews*, *2017*(76), 1485–1496. https://doi.org/10.1016/j.rser.2016.11.184

Idris, S. M., Jones, P. L., Salzman, S. A., Croatto, G., & Allinson, G. (2012). Evaluation of the giant reed (*Arundo donax*) in horizontal subsurface flow wetlands for the treatment of recirculating aquaculture system effluent. *Environmental Science and Pollution Research*, *19*, 1159–1170. https://doi.org/10.1007/s11356-011-0642-x

Jiang, D., Ge, X., Zhang, Q., & Li, Y. (2016). Comparison of liquid hot water and alkaline pretreatments of giant reed for improved enzymatic digestibility and biogas energy production. *Bioresource Technology*, *216*, 60–68. https://doi.org/10.1016/j.biortech.2016.05.052

Kam, J., Thomas, D., Pierre, S., Ashman, C., McCalmont, J. P., & Purdy, S. J. (2020). A new carbohydrate retaining variety of Miscanthus

increases biogas methane yields compared to M x giganteus and narrows the yield advantage of maize. *Food Energy Security*, *00*, e224. https://doi.org/10.1002/fes3.224

Kiesel, A., & Lewandowski, I. (2017). Miscanthus as biogas substrate–Cutting tolerance and potential for anaerobic digestion. *GCB Bioenergy*, *2017*(9), 153–167. https://doi.org/10.1111/gcbb.12330

Lachowicz, H., Wróblewska, H., Wojtan, R., & Sajdak, M. (2019). The effect of tree age on the chemical composition of the wood of silver birch (*Betula pendula* Roth.) in Poland. *Wood Science and Technology*, *53*, 1135–1155. https://doi.org/10.1007/s00226-019-01121-z

Lay, J. J., Li, Y. Y., & Noike, T. (1997). Influences of pH and moisture content on the methane production in high-solids sludge digestion. *Water Research*, *31*, 1518–1524. https://doi.org/10.1016/S0043-1354(96)00413-7)

Lewandowski, I., Scurlock, J. M. O., 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. https://doi.org/10.1016/S0961-9534(03)00030-8

Li, W., Khalid, H., Zhu, Z., Zhang, R., Liu, G., Chen, C., & Thorin, E. (2018). Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin. *Applied Energy*, *226*, 1219–1228. https://doi.org/10.1016/j.apenergy.2018.05.055

Littell, R. C., Henry, P. R., & Ammerman, C. B. (1998). Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science*, *76*, 1216–1231. https://doi.org/10.2527/1998.7641216x

Littell, R. C., Milliken, G. A., Stroup, W. W., & Wolfinger, R. D. (1996). *SAS system for mixed models*. SAS Institute Inc. https://v8doc.sas.com/sashtml/hrddoc/indfiles/55235.htm

Liu, S., Ge, X., Liew, L., Liu, Z., & Li, Y. (2015). Effect of urea addition on giant reed ensilage and subsequent methane production by anaerobic digestion. *Bioresource Technology*, *192*, 682–688. https://doi.org/10.1016/j.biortech.2015.06.034

Mangold, A., Lewandowski, I., Hartung, J., & Kiesel, A. (2019). Miscanthus for biogas production: Influence of harvest date and ensiling on digestibility and methane hectare yield. *GCB Bioenergy*, *11*, 50–62. https://doi.org/10.1111/gcbb.12584

Marchetti, R., Vasmara, C., Florio, G., & Borin, M. (2016). Biomethanation potential of wetland biomass in codigestion with pig slurry. *Waste and Biomass Valorization*, *7*, 1081–1089. https://doi.org/10.1007/s12649-016-9515-3

Monti, A., Di Virgilio, N., & Venturi, G. (2008). Mineral composition and ash content of six major energy crops. *Biomass and Bioenergy*, *32*, 216–256. https://doi.org/10.1016/j.biombioe.2007.09.012

Monti, A., & Zegada-Lizarazu, W. (2016). Sixteen-year biomass yield and soil carbon storage of giant reed (*Arundo donax* L.) grown under variable nitrogen fertilization rates. *BioEnergy Research*, *9*, 248–256. https://doi.org/10.1007/s12155-015-9685-z

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. *Italian Journal of Agronomy*, *6*(3), 152–158. https://doi.org/10.4081/ija.2011.e24

Rabii, A., Aldin, S., Dahman, Y., & Elbeshbishy, E. (2019). A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration. *Energies*, *12*, 1106. https://doi.org/10.1016/10.3390/en12061106

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. *Bioresource Technology*, *152*, 107–115. https://doi.org/10.1016/j.biortech.2013.11.004

Ruf, T., Schmidt, A., Delfosse, P., & Emmerling, C. (2017). Harvest date of *Miscanthus* x *giganteus* affects nutrient cycling, biomass development and soil quality. *Biomass and Bioenergy*, *100*, 62–73. https://doi.org/10.1016/j.biombioe.2017.03.010

SAS Institute. (2015). *SAS/STAT 14.1 User's Guide*. SAS Institute. https://support.sas.com/documentation/onlinedoc/stat/141/intro.pdf

Schmidt, A., Lemaigre, S., Ruf, T., Delfosse, P., & Emmerling, C. (2018). Miscanthus as biogas feedstock: influence of harvest time and stand age on the biochemical methane potential (BMP) of two different growing seasons. *Biomass Conversion and Biorefinery*, *8*, 245–254. https://doi.org/10.1007/s13399-017-0274-6

Shilpi, S., Lamb, D., Bolan, N., Seshadri, B., Choppala, G., & Naidu, R. (2019). Waste to watt: Anaerobic digestion of wastewater irrigated biomass for energy and fertiliser production. *Journal of Environmental Management*, *239*, 73–83. https://doi.org/10.1016/j.jenvman.2019.02.122

Singleton, V. L., & Rossi, Jr., J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Soil Survey Staff. (2014). *Keys to soil taxonomy* (12th ed.). Washington, DC: USDA-Natural Resources Conservation Service.

Surendra, K. C., & Khanal, S. K. (2015). Effects of crop maturity and size reduction on digestibility and methane yield of dedicated energy crop. *Bioresource Technology*, *178*, 187–193. https://doi.org/10.1016/j.biortech.2014.09.055

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral-detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vasmara, C., Cianchetta, S., Marchetti, R., & Galletti, S. (2015). Biogas production from wheat straw pre-treated with ligninolytic fungi and co-digestion with pig slurry. *Environmental Engineering and Management Journal*, *14*, 1751–1760. https://doi.org/10.30638/eemj.2015.186

Vasmara, C., & Marchetti, R. (2016). Biogas production from biodegradable bioplastic. *Environmental Engineering and Management Journal*, *15*, 2041–2048. http://omicron.ch.tuiasi.ro/EEMJ/

Vasmara, C., & Marchetti, R. (2018). Spent coffee grounds from coffee vending machines as feedstock for biogas production. *Environmental Engineering and Management Journal*, *17*, 2401–2408. https://doi.org/10.30638/eemj.2018.238

Velázquez-Martí, B., Meneses-Quelal, O. W., Gaibor-Chavez, J., & Niño-Ruiz, Z. (2018). *Review of mathematical models for the anaerobic digestion process*. Anaerobic Digestion. https://doi.org/10.5772/intechopen.80815

Whittaker, C., Hunt, J., Misselbrook, T., & Shield, I. (2016). How well does Miscanthus ensile for use in an anaerobic digestion plant? *Biomass and Bioenergy*, *88*, 24–34. https://doi.org/10.1016/j.biombioe.2016.03.018

Wu, W. M., Jain, M. K., de Macario, E. C., Thiele, J. H., & Zeikus, J. G. (1992). Microbial composition and characterization of prevalent

methanogens and acetogens isolated from syntrophic methanogenic granules. *Applied Microbiology and Biotechnology*, *38*, 282–290. https://doi.org/10.1007/BF00174484

Yang, L., & Li, Y. (2014). Anaerobic digestion of giant reed for methane production. *Bioresource Technology*, *171*, 233–239. https://doi.org/10.1016/j.biortech.2014.08.051

Zegada-Lizarazu, W., Salvi, S., & Monti, A. (2020). Assessment of mutagenized giant reed clones for yield, drought resistance and biomass quality. *Biomass and Bioenergy*, *134*, 105501. https://doi.org/10.1016/j.biombioe.2020.105501

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & van 't Riet, K. (1990). Modeling of the bacterial growth curve. *Applied and Environment Microbiology*, *56*(6), 1875–1881.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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