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Giant reed (*Arundo donax* L.) for biogas production: land use saving and nitrogen utilisation efficiency compared with arable crops

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Abstract

Aiming to improve the sustainability of biogas supply chains, the research for alternative feedstocks is a key issue and giant reed (*Arundo donax* L.) is a promising no-food crop to be used in anaerobic digestion. In fact, giant reed is a perennial species characterised by low nutrient requirements and is able to provide promising biogas yields. Its suitability for anaerobic digestion is influenced by harvest time, since plant characteristics vary noticeably along the season. Moreover, ensiling is a storage technique that can assure a good preservation of the biomass over time, but also influence the methane yields. Therefore, the aim of this study was to assess the suitability for biogas production of giant reed silage, according to different cutting regimes, and to evaluate the efficiency in saving land and nitrogen for fuelling biogas plants, in comparison with maize and two sorghum varieties. Methane yields per hectare (Nm^3 CH$_4$ ha^{-1}) were determined by multiplying the biochemical methane potential of each substrate by the aboveground biomass of the corresponding crop. The land use coefficient (LU), namely the land needed to fuel one kW power (ha kWe^{-1}), was calculated from the estimated methane yields per hectare. Finally, nitrogen utilisation efficiency (NUtE), which is the ratio between the estimated methane yield and the nitrogen uptake per hectare (Nm^3 CH$_4$ kgN^{-1}), was determined for each crop species and according to the harvest time and frequency of giant reed. Overall, a good suitability for ensiling was observed in giant reed. When harvested in September, the crop yielded about 9900 Nm^{3}CH$_4$ ha^{-1}, while in double harvest systems biomethane was about 12,000 Nm^{3}CH$_4$ ha^{-1}, +35% and +70% than maize and sorghum respectively. Moreover, giant reed under double harvest management was the most land-conservative option, as LU was about 0.22 ha kWe^{-1}, while in annual crops it was about 0.35 ha kWe^{-1}. The higher NUtE was observed in single harvests (up to 64 Nm^3 CH$_4$ kgN^{-1}), while double harvests showed remarkably lower values, averaging 48 Nm^3 CH$_4$ kgN^{-1}. Annual crops were less efficient, since NUtE ranged from 28 Nm^3 CH$_4$ kgN^{-1} (maize) to 40 Nm^3 CH$_4$ kgN^{-1} (fibre sorghum). In conclusion, giant reed can be an alternative for biogas making, potentially providing land and nitrogen savings compared with conventional annual crops.

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Key words: Energy crops; anaerobic digestion; silage, harvest time; biomethane; nutrient utilisation efficiency.

Acknowledgements: the research was carried out under the BIOSEA Project, funded by the Ministry of Agricultural, Food and Forestry Policies (MIPAAF), Italy. The authors wish to thank Fabio Taccini and the Centre for Agri-environmental Research (CIRAA) of the University of Pisa (Italy) for their valuable support in the field trial management.

Conference presentation: SIA XLIII Congress, Pisa, 2014.

Received for publication: 9 February 2015.
Revision received: 28 July 2015.
Accepted for publication: 7 August 2015.

Italian Journal of Agronomy 2015; 10:664
doi:10.4081/ija.2015.664

Introduction

Biogas is an increasingly important bioenergy source, characterised by a clean chemical composition and a high-energy content (Weiland, 2010; Appels *et al*., 2011). During 2013, the primary energy production from anaerobic digestion (AD) in EU a was 13.4 Mtoe (+25% from 2011), while the electricity production from biogas was equal to 52.3 TWh (+16%) (EurObserv'er Report, 2014). Biogas is relatively easier to be transported, stored and converted than other biofuels. It can be used in combined heat and power plants or upgraded to biomethane, to be used as gaseous vehicle fuel or injected into the natural gas grid (Weiland, 2010; Appels *et al*., 2011; Deublein and Steinhauser, 2011).

Several feedstocks, such as energy crops, agricultural residues, organic wastes and manures, are suitable for biogas production (Bonari *et al*., 2009; Weiland, 2010; Appels *et al*., 2011; Deublein and Steinhauser, 2011). Nevertheless, up to now the most used biomasses are originated from annual food crops, like maize and sorghum, which require relevant agronomic inputs and are not well suited to marginal soils, thus resulting in competition for land with food production (Ceotto, 2008; Murphy *et al*., 2011). On the other hand, alternative crop species can represent promising biomass sources for AD. In particular, perennial rhizomatous grasses like giant reed (*Arundo donax* L.) show some relevant advantages compared with annual crops. Giant reed is a no-food crop, capable of high aboveground biomass yields, which showed good adaptability to marginal quality land, as well as low input requirements (Angelini *et al*., 2009; Nassi o Di Nasso *et al*., 2011, 2013; Schievano *et al*., 2012). As a perennial crop, it can positively affect soil quality, since it can reduce the risk of soil erosion and increase the soil carbon content (Fagnano *et al*., 2014; Barbanti *et al.*; 2014). Moreover, it showed good efficiency in nutrient utilisation (Nassi o Di Nasso *et al*., 2013), that is particularly important since the production of fertilisers is a high-energy demanding process, and greenhouse gases emissions are caused by the application of nitrogen fertilisers (*e.g*., N_2O) (Crutzen *et al*., 2008).

Biochemical methane potential (BMP) has been widely used to assess methane yields of organic substrates converted under anaerobic conditions (Angelidaki *et al*., 2009; Raposo *et al*., 2011). Grasses and other lignocellulosic feedstock have been extensively studied as interesting biomasses for biogas production (Lehtomäki *et al*., 2008; Nizami *et al*., 2009; Massé *et al*., 2010; Kandel *et al*., 2012). Although BMP is the most important parameter, anaerobic digestion kinetics is also relevant, since a higher methane production rate leads to better yields in real-scale plants (Mähnert and Linke, 2009; Grieder *et al*., 2012). Furthermore, it is important to note that chemical composition of crops influences their biomethane production. Thus, factors like crop species, harvest time and storage system can influence the biomass quality, and its methane potential (Guo *et al*., 2010; Massé *et al*., 2010; Herrmann *et al*., 2011; Kandel *et al*., 2012; Ragaglini *et al*., 2014). In particular, the methane potential is affected by harvest time, since plant characteristics markedly vary with their development stage. For instance, the nitrogen concentration and carbon/nitrogen (C/N) ratio, the non-structural carbohydrates content (NSC) and the cell wall components are typically affected (Lehtomäki *et al*., 2008; Massé *et al*., 2010; Ragaglini *et al*., 2014). Biomass storage also affects the biomethane production and it is crucial for the sustainability of the entire biogas supply chain. Ensiling represents the typical storage system for biomass addressed to anaerobic digestion (Massé *et al*., 2010; Herrmann *et al*., 2011; Williams and Shinners, 2014). In ensiled biomass, long-term storage can be achieved by the decrease of pH, obtained through the conversion of soluble carbohydrates into lactate, acetate, propionate and butyrate, thus inhibiting the growth of detrimental microorganisms (Herrmann *et al*., 2011; Kung, 2010).

The research about alternative feedstock is crucial for targeting the objective of low-input biogas productions. Thus, the aim of this study was to assess the suitability of ensiled giant reed for biogas production and its efficiency in saving land and nitrogen consumption for fuelling biogas plants, compared with conventional annual crops.

In particular, the biomass productivity, the nitrogen uptake of the crop and BMP of giant reed silages were measured and compared with those of maize and sorghum, in order to evaluate: i) how different cutting regimes can affect the efficacy of this crop in reducing the land requirement for electricity production; and ii) how the nitrogen utilisation efficiency can vary according to the harvest time and frequency.

Materials and methods

Field experiment and samples preparation

A local ecotype of giant reed was cultivated since March 2007 in 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). The crop was established on a loam soil (Typic Xerofluvent), representative of the lower Arno River plain and characterised by a shallow water table. It was characterised by 45.3% sand, 43.3% silt, 11.4% clay, pH 7.9 (1:1 w/w), 13 g kg^{-1} organic matter (Walkey-Black), 1.2 g kg^{-1} total nitrogen (Kjeldahl), 10.8 mg kg^{-1} available phosphorus (Olsen), 131 mg kg^{-1} exchangeable potassium (Dirks and Scheffer). In autumn 2006, the soil was ploughed (depth 30-40 cm), then tilled by one pass with a double-disk harrow and one pass with a field cultivator. Giant reed was established using rhizomes with a couple of buds weighing about 500 g each (20,000 units ha^{-1}), spaced in 1 m wide rows, planted at a depth of 10-20 cm. In the establishment year, fertilisers 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 fertiliser 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 vegetative season. Weeding, irrigation, and pest control were not carried out at any point during the trial.

From 2007 to 2010 the whole experimental field was harvested once a year in winter. During the growing season of the year 2011, the 5-year old crop sprouted on the 20th of March, then it was harvested at 5 different times from June to September (A1-A5), each one replicated 3 times (15 plots). Resprouting after first cut was expected, thus leading to perform a second cut in early autumn (October 2011) from plots where crop regrowth was not negligible, that were those harvested in June and July (AR1 and AR2).

Meanwhile, maize (DKC666, FAO 600, Dekalb) (M), fibre sorghum [Biomass 133, *Sorhum bicolor* (L.) Moench, Syngenta] (S1) and forage sorghum [Jumbo, *Sorghum bicolor* (L.) Moench x *Sorghum sudanense* (Piper) Stapf, Padana Sementi] (S2) were sown on close fields (row spacing 0.75 m) with same soil conditions (May 2011) and grown according to the standard crop management of the site. In sorghum and maize, 100 kg P_2O_5 ha^{-1} (triple super phosphate) and 100 kg K_2O ha^{-1} (potassium sulphate) were applied, while different nitrogen fertilisation rates were adopted for the two crops (100 kg N ha^{-1} and 250 kg of N ha^{-1} as urea for sorghum and maize, respectively). Moreover, maize was irrigated with a total of about 200 mm, while sorghum was rain fed. Both the annual crops took advantage of chemical weed control and were harvested at wax ripeness (September 2011) (Table 1).

At harvest time, biomass fresh weight of each crop was determined by sampling a 2-m^2 area within each plot (12×3 m). Dry matter content was determined by oven drying at 65°C until constant weight, in order to assess the aboveground dry biomass yield and to verify the suitability for ensiling. Samples from the annual crops and from each harvest time of giant reed were ensiled at laboratory scale by bulking fresh biomass from the field replications, then chopped by an electric powered shredder (AL-KO 1600), treated with a microbial inoculum (11CH4, Pioneer Hi-Bred Italia) and kept into vacuum-sealed polyethylene bags for 60 days. Samples for chemical analyses and BMP determination were prepared by milling in a Retsch SM1 rotor mill equipped with a 1 mm grid, then stored at –20°C.

Chemical analyses

Total nitrogen concentration (TKN) was determined on raw biomass before ensiling according to the Kjeldahl method. Total solids (TS) and volatile solids (VS) concentrations of crops silages were determined according to standard methods (APHA, 2005).

Further analyses were carried out on ensiled giant reed only, in order to determine pH, N concentration and C/N ratio, lignin and NSC. N concentration and C/N ratio of silages were assessed by elemental analysis (Leco CHN 600). Lignin was quantified using the acetyl bromide method, absorbance was measured at 280 nm, and then lignin content was calculated using the Lambert-Beer equation (Fukushima and Hatfield, 2004). The concentration of NSC was calculated as the sum of water-soluble sugars plus starch, determined according to Giovannelli *et al*. (2011). For each sample and parameter, three technical replicates were analysed.

Anaerobic digestion

Biochemical methane potential was determined according to the methodology described by Ragaglini *et al*. (2014), resumed as follows.

Anaerobic digestion was carried out in 2 L batch reactors and the assays were conducted in triplicates on giant reed, maize and sorghum samples. Each reactor received 300 g of inoculum that was suspended in a basal mineral medium, prepared according to the ISO 11734 standard (ISO, 1995), up to a final filled volume of 1 L. Three blank experiments were also performed with 300 g of inoculum, demineralised water and minerals only. The inoculum was originated from the methanogenic stage of a mesophilic anaerobic digester, fed mainly with energy crops (maize silage), agroindustrial residues and poultry manure. The inoculum was also prepared according to the ISO 11734 standard, except for inorganic carbon removal procedure that has not been carried out (Angelidaki *et al*., 2009). The anaerobic sludge was sieved through a 1 mm mesh, then its total solids and volatile solids content were determined (147.0 g kg^{-1} and 109.6 g kg^{-1}, respectively). Before the beginning of the assay, the inoculum was pre-digested for 5 days at 37°C, aiming to degas it and to lower its concentration of readily available organic matter. In order to avoid inhibition phenomena, the substrates were added according to a ratio between the volatile solids of the inoculum and those of the substrate (VS_i:VS_{sub}) equal to 2:1 (Table 1) (Angelidaki *et al*., 2009; Raposo *et al*., 2011). Then, the reactors were sealed and flushed with N_2 and incubated at 37±1°C until biogas production became negligible (45 days). During the assay, biogas pressure in each reactor was continuously measured by pressure piezo-resistive transducers and recorded by a dedicated programmable logic controller connected to a PC, while methane concentration (%CH_4) was measured at discrete intervals by gas chromatography (micro-GC Agilent 3000). The intrinsic methane production of the inoculum was subtracted to the methane volume produced by each reactor. Then, BMP were calculated by ratio with the amount of VS_{sub} put into the reactors. Analogously, the biochemical biogas potential (BBP) was calculated by subtracting the intrinsic biogas production of the inoculum to the biogas volume produced in each reactor, then divided by VS_{sub}.

The kinetics of anaerobic digestion was examined by regression on time of the daily-cumulated methane measured in each reactor, using the five parameters Modified Gompertz function (Beuvink and Kogut, 1993; Grieder *et al*., 2012):

$$y = A \cdot exp\left[\frac{M_r}{D_r} \cdot \exp(-D_r \cdot x) - \frac{M_s}{D_s} \cdot \exp(-D_s \cdot x)\right] \qquad (1)$$

where:
A represents the upper limit;
M the initial relative growth rate;
D the relative growth rate at inflection;
r the rapid initial phase;
s the slow final phase.

Goodness of fit (R^2_{adj}, root mean square error) was assessed for each substrate. Then, the function was used to calculate four kinetics parameters: the time, expressed in days, when 50% and 95% of methane production was reached (namely T_{50} and T_{95}), the mean daily production rate from the beginning of the assay to T_{50} (R_{50}), and the mean daily production rate from the beginning of the assay to T_{95} (R_{95}). Curve fitting and model parameterisation were performed using the R software, version 3.1.2, and the *nmle* package (Pinheiro *et al*., 2013).

Land use and nitrogen utilisation efficiency

Methane yields per hectare (Nm^3 CH_4 ha^{-1}) were determined by multiplying the mean BMP of each substrate by the aboveground biomass production of the corresponding crop. Dry matter losses occurring during the ensiling process were not determined. Regarding giant reed, biomass yields from first cuts and second cuts from the same plots were summed, in order to compare the whole aboveground production of double harvest systems (namely A1+AR1, A2+AR2) with the yield obtained by single harvests (A3, A4, A5) and annual crops (M, S1, S2) (Table 1). Therefore, methane yields of first cuts and second cuts were also summed, in order to evaluate the methane productivity of double harvest systems. A land use coefficient (LU), namely the land needed to fuel one kW power (ha kWe^{-1}), was calculated from the estimated methane yields per hectare, considering the lower heating value of methane (35.8 MJ Nm^{-3}), the power conversion efficiency (η=0.3) and operating hours per year (h=8000).

Nitrogen uptakes were assessed by multiplying the TKN concentrations of the crops by the aboveground biomass yields, and the nitrogen removal levels of double harvest systems were determined by coupling the uptakes of the first and the second cuts. Nitrogen utilisation efficiency (NUtE), defined as the ratio between the estimated methane yield and the nitrogen uptake per hectare (Nm^3 CH_4 kgN^{-1}), was also determined for each crop species and according to the harvest time and frequency of giant reed. Therefore, in the present study NUtE is intended as the efficiency in using nitrogen for biomethane production, in analogy with studies considering the ratio of the overall energy output to the nitrogen uptake (de Vries *et al*., 2010).

Statistical analyses

Results referred to each substrate (*i.e*., biomass composition traits, anaerobic digestion parameters) were compared by one-way analysis of variance (ANOVA). When double harvests were assessed, the outcomes referred to the crop (*i.e*., biomass yield, methane yield per hectare, land use coefficient, nitrogen uptake and NUtE) were also compared by one-way ANOVA, and pooled values of first and second cuts were considered. When significant differences were evidenced, post-hoc comparisons were performed using the Tukey's honest significant difference (HSD) test at the 0.05 P-level. Linear correlations were evaluated, in order to point out the mutual relationships between BMP, digestion kinetics and biomass composition traits of giant reed harvested at different times.

Results

Biomass yield and composition

During spring-summer season 2011, temperatures were in line with long-term data, with maximum values, above 30°C, reached in July and August (Figure 1). Starting from April until mid-October, when giant reed regrowth were harvested, the average daily temperature was higher than 15°C. Starting from giant reed sprouting (early March) to October, total rainfall equalled 313 mm, most of which recorded during March and September. From the sowing of maize and sorghum, till harvest season, it rained nearly 72 mm. During summer, rainfall was mainly distributed in two intensive events (up to 45 mm/day) at the beginning and at the end of the season.

Statistically significant differences were observed among the yields of the considered crops and harvest treatments (Figure 2). At the first harvest time (A1), giant reed yielded nearly 23 Mg dry matter (DM) ha^{-1}. In A2 and A3, only a slight yield increase was observed with respect to A1. The slow growth conditions observed from June to early August are put in evidence by the remarkably higher yields obtained at following cuts (A4, 31 Mg DM ha^{-1}; A5, 38 Mg DM ha^{-1}) (Figure 2). Substantial crop regrowth from A1 and A2 was observed in early October (AR1, 17 Mg DM ha^{-1}; AR2, 13 Mg DM ha^{-1}), thus leading to cumulated yields of the double harvest systems of 35-40 Mg DM ha^{-1}. Yields obtained by double harvesting did not differ significantly from cutting giant reed once in September (A5), while they were significantly higher than single cuts performed in high summer (A3). At wax ripeness, maize (M), fibre sorghum (S1) and forage sorghum (S2) yielded 25, 28 and 26 Mg DM ha^{-1}, respectively. Fibre sorghum was slightly but not significantly more productive than maize and forage sorghum. However, dry biomass yields of the annual crops did not differ from those obtained by giant reed harvested in August (A3-A4).

At harvest the TS content in giant reed varied from a minimum of 46.5% in A1 to a maximum of 52.2% in A4 and the two regrowth had a dry matter content of about 49% (Table 1). The TS of annual crops was quite lower than giant reed, 34.8% in M, 37.5% in S1 and even 26% in S2. During the vegetative season, ash content decreased from 8.6% in A1 to 4.8% in A5, while regrowth showed a higher concentration, about 9%. On the other hand, concerning annual crops, maize and fibre sorghum had lower ash content (4.9% and 5.7% respectively) while forage sorghum exhibited a higher value (7.7%).

After 60 days of ensiling, the pH of M, S1 and S2 decreased at 3.5, 3.6 and 4 respectively, while in giant reed the pH was considerably higher, with values varying, according to the harvest times, from a maximum of 5.4 to a minimum of 4.3 (Figure 3A). In giant reed silages, NSC varied noticeably among the different harvest times (Figure 3A). The highest concentrations were found in substrates from late stages (A5) and in second cuts (32 mg g^{-1}), as a consequence of higher initial carbohydrate concentrations in the raw crop (up to 110 mg g^{-1}). The lowest NSC concentration was observed in silage obtained from A1 (1.4 mg g^{-1}). In general, an increasing trend along the season may be evidenced, although A4 showed a lower NSC value than A3 and A5, and the lowest carbohydrate level in the raw crop was observed in A2. As a consequence of ensiling, carbohydrates were converted to organic acids and pH was lowered. All the giant reed silages showed pH<5, except for A1. The lowest values were observed in A5 and AR2, followed by A3 (4.30, 4.44 and 4.55, respectively). A low level of variability was observed in acetyl bromide lignin concentrations, since all the values ranged between 21.0 and 23.2 (Figure 3B). A weak increase from A1 to A5 was observed, and second cuts showed slightly lower lignin values than the A5, but these differences were not statistically significant.

N concentration in giant reed silages was higher in the early stages (about 0.75%) than in later cuts (about 0.4% in A4-A5). Thus, a decreasing trend during the vegetative season was shown, although A3 had an N concentration slightly higher than A2 (Figure 3C). Second cuts were also quite rich in nitrogen (on average 0.7% in AR1-AR2). At opposite, C/N ratio was higher in A4-A5 (~125), while its lowest values were recorded in A1 and in second cuts (~70) (Figure 3D). AR2 showed a higher N concentration and a lower C/N ratio than AR1, highlighting that nitrogen decreased at the increase of plant age in second cuts as well as in first cuts.

Digestion kinetics and biochemical methane potential

In general, the Modified Gompertz function (Eq. 1) provided very good fitting between time and daily cumulative methane measured in each reactor, as already showed in previous works (Grieder *et al*., 2012; Ragaglini *et al*., 2014). In fact, the average R^2_{adj} was very close to 1 for all the considered substrates (Table 2). Therefore, this model was adopted to estimate kinetics parameters T_{50} and T_{95}. The investigated substrates exhibited significant differences in digestion kinetics (P<0.001) (Figure 4). During the first days of digestion, the methane production was quite faster in AR1 and AR2, that reached the 50% of the accumulated production (T_{50}) at about 6 days from the beginning of the assay. About annual crops, it was observed that T_{50} occurred at about 8 days, with no remarkable difference between maize and sorghum

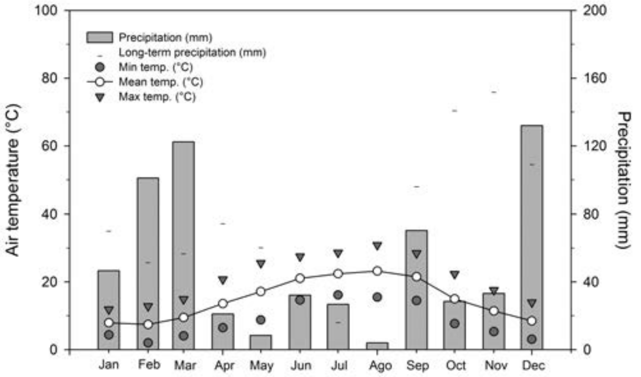

Figure 1. Monthly and long-term precipitation, and minimum, maximum, mean air temperature in San Piero a Grado (Pisa, Italy) for the year 2011. The graph is presented as a Bagnouls and Gaussen diagram, in order to identify dry months (when precipitation is equal to or less than twice the monthly mean air temperature value, P≤2T) (Bagnouls and Gaussen, 1957).

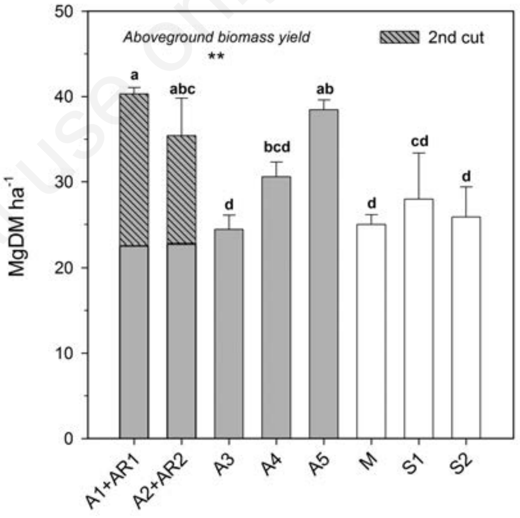

Figure 2. Aboveground biomass yield of giant reed and annual crops silages. Significance levels of ANOVA are shown: *P<0.05, **P<0.01, ***P<0.001. Different letters are for statistically different means at the P<0.05 level.

Table 1. Harvest date, total solids content on the fresh matter, volatile solids and ash concentration on the dry matter of giant reed and annual crops silages.

Biomass	Harvest date	TS (% of FM)	Ash (% of DM)	VS (% of DM)
A1	21-Jun	46.5	8.6	91.5
A2	15-Jul	49.2	6.1	93.9
A3	02-Aug	51.9	6.7	93.3
A4	22-Aug	52.2	6.5	93.5
A5	20-Sep	51.7	4.8	95.2
AR1	18-Oct	48.9	9.0	91.0
AR2	18-Oct	48.6	8.9	91.1
M	31-Aug	34.8	4.9	95.1
S1	12-Sep	37.5	5.7	94.3
S2	12-Sep	25.9	7.7	92.3

TS, total solids content; FM, fresh matter; VS, volatile solids; DM, dry matter; A1-A5, first cuts of giant reed; AR1-AR2, second cuts of giant reed; M, maize; S1, fibre sorghum; S2, forage sorghum.

varieties. Harvest times from A1 to A3 reached the 50% of the methane production during the 9-10th day, while in A4 and A5 the early phase of digestion was slower and T_{50} was reached only at the 11-12th day. A similar pattern was found for T_{95}, since second cuts reached the 95% of the cumulative production in about 21days, while the highest values were found in A4 and A5 (37-38 days). Intermediate values were observed in maize (31 days), sorghum (32 days) and in first cuts of giant reed (A1-A3, >35 days).

After 45 days, the highest BMP were measured in AR2, M and AR1 (414.4, 370.5 and 365.6 NL kgVS^{-1}, respectively) (Table 3). A1, A2 and A3 showed intermediate values (309.0, 323.0 and 332.5 NL kgVS^{-1}, respectively), while later giant reed cuts and the two sorghum varieties exhibited significantly lower potentials, ranging 273.1-286.3 NL kgVS^{-1} ($P<0.001$). In particular, the lowest value was measured in A5, about -34% compared with AR2 and 26% compared with M. On average, the BMP of giant reed silages was 289 NL kgVS^{-1} at first cut, while it was 390 NL kgVS^{-1} at second cut. The highest BBP was that of AR2 (682.9 NL kgVS^{-1}), followed by M and AR1 (648.9 and 611.7 NL kgVS^{-1}, respectively), while the lowest BBPs were measured in fibre and forage sorghum (about 410 NL kgVS^{-1}) (Table 3).

The digestion rate from the beginning of the assay to T_{50} (R_{50}) was higher in the most productive substrates, that were AR2 and AR1 (about 30 NL kgVS^{-1} day^{-1}), followed by M (22 NL kgVS^{-1} day^{-1}). Interestingly, the two sorghum varieties showed quite high R_{50} values. In particular, fibre sorghum (S1, 18 NL kgVS^{-1} day^{-1}) was not significantly different from early cuts of giant reed (A1-A3, 17-19 NL kgVS^{-1} day^{-1}), while lower rates were observed in later cuts (A4-A5, 12-14 NL kgVS^{-1} day^{-1}). Results were substantially similar for the digestion rates from the beginning of the assay to T_{95} (R_{95}), although significant differences were shown between AR2 and AR1, while the R_{95} of forage and fibre sorghum did not differ from those of A4-A5.

Table 2. Values of R^2_{adj} and root mean square error for the modified Gompertz equations fitted for each substrate.

	R^2_{adj}	RMSE
A1	0.9985	3.12
A2	0.9989	2.90
A3	0.9989	2.95
A4	0.9990	2.45
A5	0.9988	2.73
AR1	0.9994	2.16
AR2	0.9997	1.63
M	0.9991	2.82
S1	0.9953	5.00
S2	0.9949	5.31

RMSE, root mean square error; A1-A5, first cuts of giant reed; AR1-AR2, second cuts of giant reed; M, maize; S1, fibre sorghum; S2, forage sorghum.

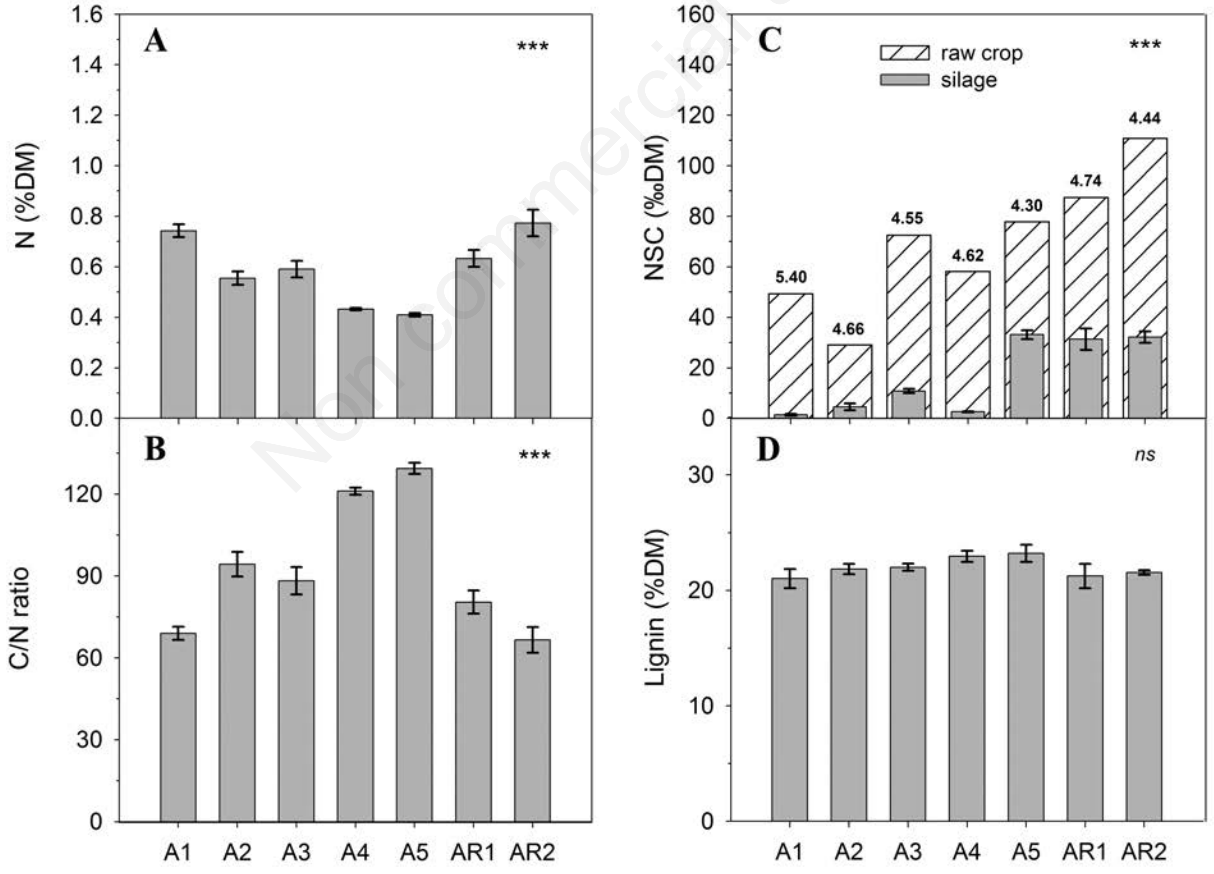

Figure 3. Giant reed chemical traits according to different cuts. A) Non-structural carbohydrates (NSC) (shaded bars for the raw crop, silage pH is reported over the bars); B) Acetyl bromide lignin; C) Nitrogen concentration; D) Carbon/nitrogen (C/N) ratio. Raw crop NSC concentrations are from Ragaglini *et al.* (2014). DM, dry matter; ns, not significant;*P<0.05, **P<0.01, ***P<0.001.

Regarding giant reed silages, some correlations between composition and digestion parameters were observed (Table 4). In particular, N concentration in biomass after ensiling was positively correlated with BMP and with pH, while it showed negative correlations with kinetic parameters T_{50} and T_{95}. For the C/N ratio, opposite correlations were found. NSC concentration was positively correlated with the biogas methane content (%CH_4), while it was negatively correlated with pH, T_{50} and T_{95}. Longer digestion times led to generally lower methane potentials, as showed by negative correlations between T_{50}-T_{95} and BMP. Lastly, the biogas methane content (%CH_4) exhibited negative correlations with pH and T_{95}.

Methane yields per hectare and land use savings

Considering first cuts (A1, A2) and single cuts (A3, A4, A5) of giant reed, despite the highest BMP was reached in A3, methane yields per hectare were higher in late harvests, as a consequence of the higher aboveground biomass production. In fact, A5 was the most productive single cut of giant reed, exceeding 9900 Nm^3CH_4 ha^{-1} (Figure 5A). Annual crops showed lower potentials: maize reached about 8800 Nm^3CH_4 ha^{-1}, while the two sorghum varieties were less productive (S1, 7625 Nm^3CH_4 ha^{-1}; S2, 6555 Nm^3CH_4 ha^{-1}). These values were in line with those achieved by giant reed in A3 and A4. Under double harvest systems, giant reed substantially exceeded those production levels, achieving about 12,200 and 11,700 Nm^3CH_4 ha^{-1} in A2+AR2 and A1+AR1, respectively. Those methane yields were about +22% and +17% higher than A5.

Methane potential yields per hectare have a direct impact on the land required to provide the feedstock for biogas plants. LU for giant reed and annual crops are reported in Figure 5B. The highest LU were calculated for forage sorghum (0.41 ha kWe^{-1}), while fibre sorghum showed values similar to A3 (0.35 ha kWe^{-1}). Moreover, slightly lower values were calculated for maize (0.30 ha kWe^{-1}), while giant reed harvested in A5 required 0.27 ha kWe^{-1}. However, the most land-conservative choices were A2+AR2 and A1+AR1, needing about 0.22 ha kWe^{-1}.

Nitrogen uptakes and nitrogen utilisation efficiency

Compared with single late harvests, the nitrogen uptake of giant reed was markedly raised by double harvests, owing to increased TKN concentrations of both first and second cut (Figure 6A). In particular, A1+AR1 caused the removal of 280 kgN ha^{-1}. However, an even higher nitrogen uptake was observed in maize (320 kgN ha^{-1}), as it was the product of higher TKN concentrations (about 1.3%) and yields of about 25 Mg DM ha^{-1}. Also A2+AR2 showed a high level of nitrogen consumption (225 kgN ha^{-1}), while no significant differences were observed among A3, A4 and A5 that removed on average 145 kgN ha^{-1}. Forage and fibre sorghum did not differ significantly between each other and their nitrogen uptake was about 190 kgN ha^{-1}, as result of relatively low N concentrations (about

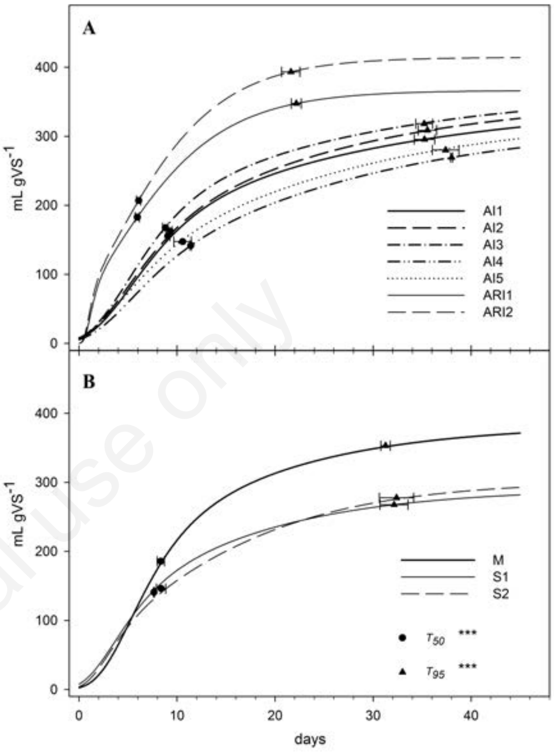

Figure 4. Anaerobic digestion kinetics of: A) giant reed silage obtained at different times (A1-A5, first cuts; AR1-AR2, crop regrowth); B) silages from annual crops (M, maize; S1, S2; sorghum). T_{50} (●), T_{95} (▲) and their standard deviations (horizontal bars) are reported. Significance levels of ANOVA for T_{50} and T95 are shown: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Table 3. Biochemical methane potential (mL gVS^{-1}) and mean daily methane production rate (mL gVS^{-1} day^{-1}) from the beginning of the assay to T_{50} (R_{50}) and to T95 (R_{95}).

	BMP***	BBP***	R50***	R95***
A1	309.0 (29.3)^{cde}	534.6 (47.4)^{de}	17.15 (1.04)^{cd}	8.41 (1.14)^{def}
A2	323.0 (15.4)^{cd}	561.3 (26.9)^{cd}	17.46 (0.91)^{cd}	8.71 (0.80)^{de}
A3	332.5 (16.3)^{bc}	582.2 (21.6)^{bcd}	19.09 (1.83)^{bc}	9.05 (0.69)^{d}
A4	280.3 (4.7)^{e}	476.7 (14.9)^{ef}	12.43 (0.07)^{e}	7.09 (0.09)^{f}
A5	273.1 (32.9)^{e}	449.3 (56.0)^{fg}	14.02 (1.23)^{de}	7.49 (0.15)^{ef}
AR1	365.6 (11.7)^{b}	611.7 (15.3)^{bc}	30.86 (2.86)^{a}	15.70 (0.91)^{b}
AR2	414.4 (43.8)^{a}	682.9 (87.7)^{a}	33.99 (2.09)^{a}	18.17 (0.91)^{a}
M	370.5 (24.2)^{b}	648.9 (34.4)^{ab}	22.44 (3.09)^{b}	11.30 (0.89)^{c}
S1	281.9 (6.3)^{e}	410.2 (8.0)^{fg}	18.41 (0.60)^{c}	8.34 (0.34)^{def}
S2	286.3 (14.5)^{de}	407.8 (12.7)^{g}	17.50 (0.99)^{cd}	8.60 (0.86)^{def}

BMP, biochemical methane potential; BBP, biochemical biogas potential; A1-A5, first cuts of giant reed; AR1-AR2, second cuts of giant reed; M, maize; S1, fibre sorghum; S2, forage sorghum. Significance levels of analysis of variance and Tukey's honest significant difference test results are shown: *$P<0.05$, **$P<0.01$, ***$P<0.001$. Different letters are for statistically different means at the $P<0.05$ level, standard deviations are reported in brackets.

0.7%). The higher nitrogen uptake of double harvest systems was only partially compensated by larger methane yields, as evidenced by their nitrogen utilisation efficiencies (Figure 6B). A1+AR1 yielded 45 Nm^3 CH$_4$ kgN^{-1} that was significantly the lowest NUtE observed in giant reed, while the nitrogen utilisation efficiency of A2+AR2 did not significantly differ from that of A3 (52 Nm^3 CH$_4$ kgN^{-1}). Single late harvests showed the highest efficiencies, ranging from 64 Nm^3 CH$_4$ kgN^{-1} (A5) to 60 Nm^3 CH$_4$ kgN^{-1} (A4). On the contrary, annual crops were markedly less efficient in nitrogen utilisation: M and S2 yielded 28 and 35 Nm^3 CH$_4$ kgN^{-1} respectively, while S1 showed a NUtE lower but not significantly different than that of A1+AR1 (40 Nm^3 CH$_4$ kgN^{-1}).

Discussion

In this study, an alternative crop for biogas purposes (*i.e.*, giant reed) was compared with conventional annual crops (*i.e.*, maize, fibre sorghum and forage sorghum), aiming to evaluate their suitability for anaerobic digestion, and lastly their land use and nitrogen utilisation efficiency. Maize is a widespread crop for biogas production especially where intensive, husbandry-related farming systems are established. In fact, high yields in modern maize hybrids are related to fertile soils and relevant fertiliser and energy inputs. On the other hand, good pro-

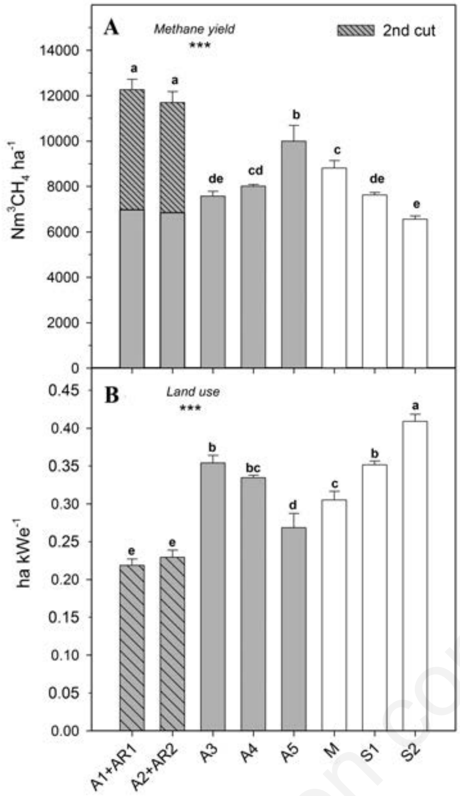

Figure 5. A) Methane yield per hectare and B) land use coefficient (ha kWe^{-1}) of giant reed and annual crops silages. Significance levels of ANOVA are shown: *$P<0.05$, **$P<0.01$, ***$P<0.001$. Different letters are for statistically different means at the $P<0.05$ level.

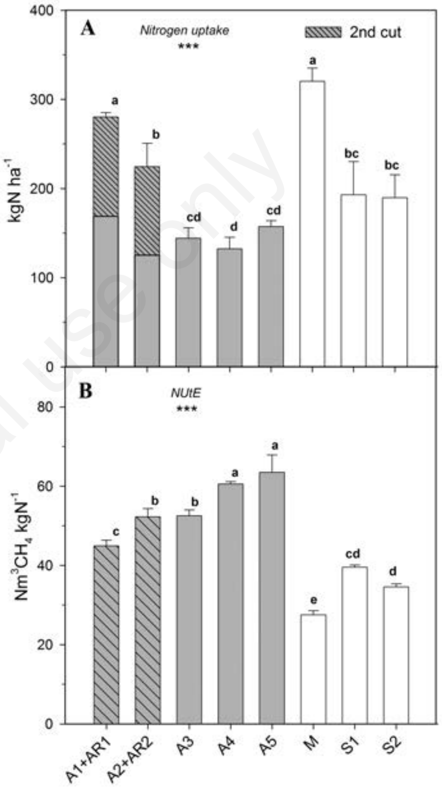

Figure 6. A) Nitrogen uptake and B) nitrogen utilisation efficiency (NUtE) of giant reed and annual crops silages. Significance levels of ANOVA are shown: *$P<0.05$, **$P<0.01$, ***$P<0.001$. Different letters are for statistically different means at the $P<0.05$ level.

Table 4. Pearson's correlation matrix between some characteristics of giant reed feedstocks and anaerobic digestion parameters.

	N	C/N	NSC	BMP	%CH$_4$	pH	T_{50}
C/N	–0.98***	-	-	-	-	-	-
NSC	0.03 ns	0.02 ns	-	-	-	-	-
BMP	0.78***	–0.77***	0.37 ns	-	-	-	-
%CH$_4$	0.02 ns	0.10 ns	0.75***	0.38 ns	-	-	-
pH	0.43*	–0.45*	–0.53*	–0.09 ns	–0.53*	-	-
T_{50}	–0.72***	0.76***	–0.53*	–0.85***	–0.30 ns	–0.03 ns	-
T_{95}	–0.62**	0.63**	–0.63**	–0.83***	–0.46*	0.08 ns	0.94***

N, nitrogen; C/N, carbon/nitrogen ratio; NSC, non-structural carbohydrates contents; BMP, biochemical methane potential; T_{50}, T_{95}, the time, expressed in days, when 50% and 95% of methane production was reached. Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$, ns, not significan.

ductivity under low input conditions has been observed in most sorghum varieties (Zegada-Lizarazu and Monti, 2012), as well as in giant reed (Nassi o di Nasso *et al*., 2013). For these reasons, maize was cultivated under a high input regime; at opposite, the other crops took advantage of much lower fertilisation levels (250 *vs* 100 kgN ha^{-1}) and they were rainfed. Biomass yields of maize, fibre and forage sorghum were in line with those observed by other authors under similar conditions (Barbanti *et al*., 2014), while the yield of sorghum could be substantially increased by irrigation (Roncucci *et al*., 2014). Regarding the growth of giant reed as assessed by cutting at different times, a modest yield increase was recorded from June to early August, since the crop was presumably limited by water deficit, while the growth was intense afterwards. In fact, giant reed is able to persist in environments in which seasonal drought occurs and to recover from water scarcity when stressful condition are mitigated, by means of large carbohydrate reserves in the rhizomes (Mann *et al*., 2013). The aboveground biomass in giant reed peaked at a quite high value in September (A5), comparable with those obtained when irrigated or grown in fertile soils (>30 Mg ha^{-1}) (Angelini *et al*., 2009; Mantineo *et al*., 2009; Nassi o di Nasso *et al*., 2011).

At our knowledge, no batch tests on anaerobic digestion of giant reed silages have been reported until the present study. Similar BMPs were observed on the unensiled biomass at different harvest times of giant reed (Ragaglini *et al*., 2014). Other authors focused on this crop harvested in early autumn, reporting generally lower values (about 220-270 NL kgVS^{-1}) (Di Girolamo *et al*., 2013; Barbanti *et al*., 2014). By literature, BMPs obtained from maize silages were found to range from about 310 NL kgVS^{-1} to 370 NL kgVS^{-1} (Bruni *et al*., 2010; Klimiuk *et al*., 2010; Barbanti *et al*., 2014), thus being in line with the results of the present study. Compared to maize, lower BMP values are generally found in fibre and forage sorghum varieties, usually between 260 and 300 NL kgVS^{-1} (Sambusiti *et al*., 2013; Barbanti *et al*., 2014). However, a wide range of values for the annual crops is reported, as a consequence of the large variability of silage characteristics, according to variety, maturity class, harvest time and climatic conditions.

Particular relevance should be given to the second cuts of giant reed. The dry aboveground biomass from first and second cuts substantially matched that of a single cut performed in September (Figure 2). Thus, the increased methane yields obtained by double harvest systems (Figure 5) depended mostly on the high BMPs shown by the second cuts. Also other perennial grasses, such as switch grass and reed canary grass, gave higher methane potentials when harvested multiple times (Massé *et al*., 2010; Kandel *et al*., 2012). Moreover, second cuts exhibited noticeably high digestion rates, that is a relevant aspect in real-scale continuously fed digesters, since digestion kinetics affects the retention time of the substrates (Mähnert *et al*., 2009; Grieder *et al*., 2012). In general, better digestion rates are observed in annual crops compared with perennial crops and in maize compared with sorghum hybrids, in substantial agreement with the results of this study (Klimiuk *et al*., 2010; Barbanti *et al.*, 2014).

According to the most of the literature, suitable feedstocks for biogas production should have high concentrations in NSC, which are easily fermented, and low levels of hemicelluloses and lignin, which are poorly degradable (Deublein and Steinhauser, 2011). Nevertheless, a correlation between BMP and NSC was not evidenced in giant reed silages, while higher concentrations of degradable carbohydrates were related to reduced digestion times, and thus to more favourable kinetics. These results are partially in line with those previously reported by Ragaglini *et al*. (2014), who did not observe any correlation between NSC, BMP and kinetics on unensiled giant reed. A marginal role of these components might be hypothesised also in this case, since NSCs in silages were markedly lower than in the raw crop (Figure 3A). Thus, the digestion performances were probably more influenced by other factors. Although no significant variations were found in lignin of giant reed silages, as well as in the raw crop (Ragaglini *et al*., 2014), crop maturity is known to affect negatively the digestibility of grasses through several mechanisms (*e.g.*, modifications of cellulose crystallinity, lignin polymerisation and composition), thus a decline of bioavailability of cellulose over the season can even so be inferred (Nizami *et al*., 2009; Monlau *et al*., 2013). Conversely, increased bioavailability may be recognised as a positive factor and the concurrence of high NSC and high cellulose bioavailability at early stages may explain the positive correlation.

Feedstocks obtained from crop regrowth showed similar nitrogen concentrations and C/N ratios to those originated from early cuts. Nitrogen concentration decreased over time, while C/N ratio showed an opposite trend, as results of carbon accumulation, nitrogen relocation from the aerial parts to the rhizome and by leaf loss triggered by senescence, in line with previous findings (Kandel *et al*., 2012; Nassi o di Nasso *et al*., 2013). As observed by Ragaglini *et al*. (2014), double cutting prevented the crop from senescing and allowed to harvest plants having a higher proportion of leaves, which are typically more digestible than the stems. In these terms, C/N ratio and N concentration may contribute to explain the enhanced digestibility observed in giant reed silages obtained from double harvesting.

Ensiling can have a moderate pre-treatment effect on biomass, thus potentially improving the bioavailability of cellulose (Herrmann *et al*., 2011). Nevertheless, comparing the BMPs of giant reed silage with those of the raw biomass (Ragaglini *et al*., 2014), ensiling did not seem to have substantially increased the digestion performances. Therefore, estimated methane yields per hectare from giant reed silage were substantially in line with those previously reported by Ragaglini *et al*. (2014). However, carbohydrates are partially fermented during silage making, causing dry matter and energy losses (Herrmann *et al*., 2011; Williams and Shinners, 2014) that were not considered in this study. For all these reasons, definitive conclusions on the ensiling of giant reed cannot be drawn and further research should be addressed on these aspects.

Among all the considered alternatives, double harvests and single late harvests of giant reed gave the highest methane outputs, as a consequence of maximised biomass yields. In fact, Schievano *et al*. (2012) compared maize (yielding about 20 Mg DM ha^{-1}) with giant reed harvested once in September and reported comparable results (6750 *vs* 11,280 Nm^{3}CH$_4$). At opposite, Barbanti *et al*. observed poorer levels in giant reed (4900 Nm^{3}CH$_4$), due to lower yield and BMP, while the methane output of maize and fibre sorghum B133 were similar to those of the present study (about 8400 and 7500 Nm^{3}CH$_4$, respectively).

The theoretical land use of each crop, expressed as the required area to fuel 1 kWe, derives directly from the methane yields per hectare. Therefore, the less productive crop (*i.e*., forage sorghum) should require more land to fuel a power plant, while the most productive one (*i.e*., giant reed under double harvest systems) is expected to be the most preservative. For instance, a biogas plant with an installed capacity of 500 kWe would require about 100 ha of giant reed managed under a double harvest system, or about 120 ha of the same crop cut once in September, or about 135, 160, 185 ha of maize, fibre sorghum and forage sorghum, respectively. Overall, it could be stated that harvesting giant reed before the full biomass potential is achieved may lead to marginal gains in land saving, unless the crop is cut early enough to allow its resprouting. Alternatively, harvesting close to the full biomass potential should also lead to reduce land requirements. At opposite, a steep decrease in methane potentials can be expected at autumn and winter harvests (Candoni *et al*., 2014), when giant reed slows its growth and undergoes senescence (Nassi o Di Nasso *et al*., 2011).

Despite the greater methane yields, the methane production per unit of nitrogen removed was markedly lower in double harvest than in single harvest systems. Performing a first cut in June and a second cut in October removed +30% of nitrogen per unit of methane than a single cut carried out at the end of the vegetative season. Moreover, the difference between the most efficient annual crop (*i.e*., fibre sorghum) and the least efficient harvest system of giant reed (*i.e*., double harvest) was modest. In fact, fibre sorghum required just +12% of nitrogen per methane unit than A1+AR1. Therefore, the highest digestibility observed in double harvest systems was obtained at the expense of greater nutrient consumption. Interestingly, harvesting giant reed at the end of the vegetative season led to the highest nitrogen utilisation efficiency along with relevant land savings. The increase in nitrogen requirements observed on double harvests could lead to intensify the cropping system. However, digestate could be returned the soil, thus allowing to partially closing the nitrogen cycle at farm scale (Bauer *et al*., 2010; Herrmann, 2013). Furthermore, this kind of cropping systems could be proposed when high levels of nutrient removal are environmentally beneficial, such as in phytodepuration (Borin *et al*., 2013; Ciccolini *et al*., 2013). However, higher nutrient uptakes and increased consumption of rhizome reserves may lead to reduced crop vitality over time (Slewinski *et al*., 2012). Thus, the long-term regrowth capacity and the economic duration of the crop under double harvest management should be assessed.

Conclusions

Giant reed has a relevant potential for biogas production, since the ensiled crop showed good suitability for anaerobic digestion, both in terms of BMP and of digestion kinetics. According to the harvest time and frequency, relevant differences were found. Double harvest systems can potentially increase the methane yield per hectare by about 17-22% compared with the most productive single harvest time that yielded about 9900 Nm^3CH_4 ha^{-1}. Such productivity increase was mostly a consequence of the high methane potential achieved by second cuts (on average 390 NL $kgVS^{-1}$). On the contrary, annual crops showed lower methane yields, ranging from 8800 Nm^3CH_4 ha^{-1} in maize, to 7600 Nm^3CH_4 ha^{-1} in fibre sorghum and 6600 Nm^3CH_4 ha^{-1} in forage sorghum. Subsequently, giant reed could allow considerable land use saving compared with annual crops conventionally grown for biogas purposes. In fact, giant reed showed a land demand of 0.22 ha kWe^{-1} under double harvest systems, while 0.27 ha kWe^{-1} are required when a single late harvest was carried out. On the contrary, higher values were observed for annual crops (about 0.30 ha kWe^{-1}) and harvesting once in summer did not lead to any relevant advantage compared with annual crops, since the land consumption was 0.33-0.35 ha kWe^{-1}, slightly higher than that of maize. Nitrogen utilisation efficiency was also improved when giant reed silages were used for biogas production, being consistently higher than that of conventional crops. In our environment, the best efficiency was reached by a single harvest in September (64 Nm^3 CH_4 kgN^{-1}), while double harvest systems led to poorer results (48 Nm^3 CH_4 kgN^{-1}).

In conclusion, the estimated land use and nitrogen utilisation efficiency could be used to assess the sustainability of biogas supply chains. According to these parameters, two most promising giant reed-based options could be hypothesised, relying on: i) double harvest, that minimised the land use, while nitrogen utilisation efficiency was relatively low; ii) single late harvest, that maximised the nitrogen utilisation efficiency, exhibiting a moderate land use. The first scenario could be viable in nutrient-rich environments, while the latter could be related to lower fertility conditions. Nonetheless, further investigations are advisable, since other environmental, economic and logistic aspects can strongly affect the sustainability of the biogas supply chain.

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